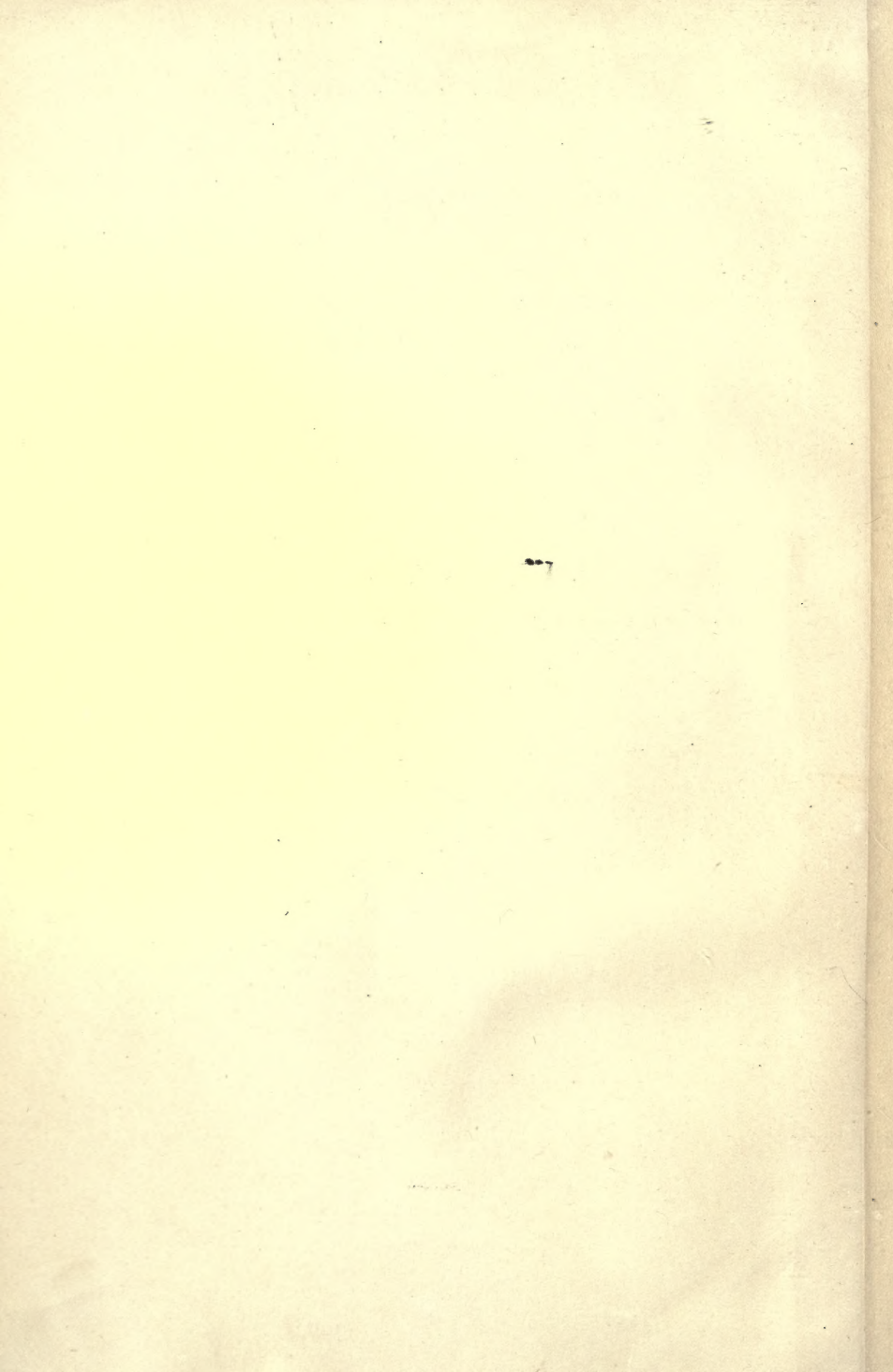# PHYSIOLOGY OF PLANTS

PRACTICAL TEXT-BOOK

OF

PLANT PHYSIOLOGY

BY

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WITH ONE HUNDRED AND FIFTY-NINE ILLUSTRATIONS.

PREFACE

THE extension of knowledge in all departments of botany during the last few years has widened the application of the principles of physiology, and altered the relative importance of many phases of the subject. The most notable development consists of the universal recognition of the underlying and basal importance of the irritability of the organism ; "that there is no vital process which is uninfluenced by stimuli, which permit, cause, restrict, or regulate the particular action in question." A second conception of no less importance is that of the intricate correlation existing between all of the activities of the vegetal organism, and the reciprocal interaction of all physiological stimuli.

The arrangement of the subject in the following pages is an effort to place before the student a method by which a working knowledge of the physiological complex of the plant may be acquired. The disposition of the subject matter entailed by this treatment consists, briefly stated, in the study of the particular functions and properties of the organism, in connection with the agencies and forces which influence or initiate them, and a consideration of the general processes of plant life. To this end the first portion of the book, inclusive of chapters I.–VII., is devoted chiefly to the special forms of irritability exhibited by typical organisms, and the second part is taken up with a more critical consideration of the broader phases of the activity of the plant : a treatment of the subject well adapted to the convenience of the independent worker, and to the exigencies of instruction.

A discussion of the principles of the subject is interwoven with the directions for practical demonstrations in order to afford means of interpretation of the experimental results secured : such discussion is naturally limited to the statement of prevalent generalizations in greater part ; the space at command does not permit a

critical presentation of all of the aspects of any part of the sub-
ject. The chief purpose of the author is to present practical di-
rections for the demonstration of the principal phenomena of the
physiology of the plant, and also details of experimental methods
suitable for the exact analyses requisite in research work. Cita-
tions of literature have been made by no single fixed rule. In
some instances reference is made to the most important, or recent
papers, or those which treat some phase of the subject not touched
upon in the present volume, or to those which give more detailed
methods of experimentation, or to those which suggest questions
needing further investigation. The appearance and translation of
the splendid treatise of Pfeffer renders the use of more space for
either discussion, or citation unnecessary. But little attention has
been given to the definition of terms, except when demanded by
conflicting usage, and by the introduction of a few new expressions.

It is impossible to make any general survey of the subject
without being impressed with the constantly increasing amount
of attention which the physiology of plants is receiving in botanical
instruction, and the additions being made to the facilities for re-
search in this department of science. The increase in both direc-
tions has been most marked in America.

The labor of preparation of the present volume has been
materially lessened by the cordial coöperation of a number of
botanists and physiologists, which cannot be adequately acknowl-
edged here. Dr. C. C. Curtis, of Columbia University, has
revised a number of chapters of the manuscript, verified some of
the newer method outlined, and made numerous valuable sug-
gestions from the results of his own researches, and extensive
laboratory practice ; Mr. J. E. Kirkwood, of Syracuse University,
and Dr. W. J. Gies, of the College of Physicians and Surgeons,
Columbia University, prepared the experimental directions for the
chemical analysis of the body of the plant as given in Chapter
IX., and read proof of the same and other sections of the book ;
Professor Geo. E. Stone, of Massachusetts Agricultural College,
outlined some of the experimental work upon the relations of

electricity to plants, and revised the entire chapter dealing with that subject ; Dr. R. H. True, of Harvard University, has aided materially with suggestions in regard to the chapter dealing with the relations of plants to chemicals ; Mr. A. F. Woods, of the Division of Vegetable Pathology and Physiology, U. S. Department of Agriculture, kindly furnished me with results of investigations of his own, and other members of the staff, in advance of their publication ; Dr. H. M. Richards, of Barnard College, Columbia University, compiled and corrected the greater part of the appendix ; I am also indebted to Dr. G. T. Moore, of Dartmouth College, Professor Francis E. Lloyd, of Teachers College, Columbia University, and to my colleagues in the New York Botanical Garden for valued assistance. In justice to these contributors, it is to be said that revision of the final proofs has rested with the author, who holds himself responsible for the contents of the entire volume.

THE AUTHOR.

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Physiology of Plants

I. NATURE AND RELATIONS OF AN ORGANISM

1. The Constitution of Living Matter. The properties of any
mass must depend upon those of its constituents. Living mat-
ter is composed chiefly of carbon, hydrogen, oxygen, and nitro-
gen, while sulphur and phosphorus are essential constituents in
smaller proportions. A chemical and physical examination of
these substances shows that they exhibit the most widely dissimilar
characteristics. Thus carbon exhibits a greater degree of atomic
cohesion than any other known element, and may be liquefied
and volatilized only at extremely high temperatures, while oxygen,
hydrogen and nitrogen are gaseous at ordinary temperatures and
undergo liquefaction and solidification only at very low tempera-
tures. Oxygen displays the greatest range of chemical affinity
and intensity. Hydrogen and carbon have a low chemical inten-
sity and a very narrow range of chemical affinity, while nitrogen
is inert. Carbon, sulphur and phosphorus undergo allotropic
modifications, and some of the oxides present in living matter are
isomeric. The union of elements of such varying properties gives
protoplasm a molecular mobility and chemical activity that en-
ables it to undergo the changes in the arrangements of its parts
constituting *development*, with great readiness. Furthermore any
incident force falling upon substances of such great dissimilarity in
chemical activity must give rise to many kinds of transformations of
energy constituting the *functions*, each capable of infinite modifica-
tion ; as if a number· of bars of different sizes and of different
kinds of metal were suspended freely and all should be struck by

a sounding iron.   Each metal would give its sound of characteristic quality and the bars of different length of the same metal would give a different note.

2. Arrangement of the Components of Protoplasm.   The elements which enter into living matter are built up into a number of groups of compounds of which the proteids are the more important.   Associated with these are a number of others such as carbohydrates, acids, and mineral salts, which may or may not actually enter into the composition of the protoplast.   On the basis of a crude physical classification these substances may be roughly divided into diffusible crystalloids and non-diffusible colloids.   Starting from such classification protoplasm may be considered as a mass of soluble and insoluble colloids saturated by crystalloids in solution, some of which are disintegrating agents, acting upon both colloids and crystalloids, the products of decomposition and decomposable substances of both groups, and also various compounds in process of rearrangement by the synthetic activity of colloidal portions, by means of energy transformed from chemical and radiant sources (See chemical and physiological properties of the cell).

The interactions of a mass of living matter of this general structure give rise to several series of transformations or manifestations, constituting the functions of which growth, absorption, secretion, fermentation, nutrition, respiration, and movement are the principal types.   Stated in another form, the protoplast is composed of several more or less simple machines each with its own characteristic activity or motion.   These machines are not independent, but interlock as if a cog or wheel in one also formed a portion of a second or third mechanism, which in turn has other interlocking devices.   The nature of such interrelations is extremely various.   Thus certain of the machines stand side by side and interlock at one point only forming a series, which may engage with a second series at one, or every possible point of contact.   Any modification of the activity of one of these machines is necessarily communicated to all of the others interlocked with

it as manifested by alterations in the performance of function of the series (See correlations).

It is to be said that the morphological and physiological performances of the complex protoplasmic machine may be moderated, or totally suppressed by the action of incident forces, and latent capacities may be called into action, but such variation in external conditions may not originate processes, or set up action of a new kind, except by long continued influence of such force upon a great number of masses of living matter standing in a linear relation to each other. This inertia, or essential characteristic, of protoplasm is incapable of chemical or physical analysis, and the organism has acquired properties other than those due simply to its physical and chemical composition.

3. Environmental Conditions. The performance of the collective functions of living matter depends upon, or is influenced by, the presence of certain external conditions or *trophic* forces, such as temperature, light, electricity, moisture, and chemical composition of the surrounding medium or substratum. To these forces protoplasm sustains a double relationship.

First it is to be said that each of the necessary trophic environmental conditions must be present, in a certain proportion, or intensity, in order to give rise to, or to allow, the full molecular activity of the constituents, and the manifestations constituting the phenomena of the full cycle of life. Thus a certain amount of moisture is necessary to dissolve and dilute the crystalloids and soluble colloids in order that the peculiar forms of activity necessary for growth, respiration and metabolism in general may be carried on, while a certain degree of temperature is also a prerequisite for the characteristic molecular motion on which these phenomena are based. This connection of external forces with protoplasm may be designated as the *tonic* relation. Unfavorable intensity or concentration of any of the incident forces may inhibit the functions, singly or in groups, until but a residuum of activity is shown, while total suppression or undue increase of any force may bring the whole mechanism to a standstill, or state of

rigor. These extreme variations in the environment may, or may not, be followed by death. Thus spores of bacteria, and seeds have been subjected to a temperature of liquid hydrogen ($-252°$ C.) and when restored to normal temperatures resumed their functions in full. This experience invalidates the older conception of protoplasm as a substance essentially and indispensably in a constant state of adjustment to its environment, since it is impossible to estimate any molecular motion at the low temperatures named. As a matter of fact the adjustments or transformations of protoplasm may be all reduced or totally inhibited, and it may still retain its definite character.

Secondly it is to be said that rapid changes in the incident conditions induces variations in the performance of the functions, or morphological activities of living matter. The amount of such change does not bear a direct proportion to the amount of the incident force received by the living matter, and in certain instances may be directly inverse to it. This relationship has to do wholly with the extra-chemical and physical organization of protoplasm, and constitutes *irritability*. Irritability is that property of living matter by which it responds to an impinging force by the release of an amount of energy disproportionate in intensity and range of molecular motion, and is fairly illustrated by the mechanism of a rifle, or engine in which an enormous power may be released by a simple pull on a trigger or lever. The energy set free by the impinging force constituting the *stimulus*, may be manifested by alterations in the functions or by alteration, suppression, or multiplication of the organs of the plant, according to the transformations set up in the organism (10).

Trophic forces may act with such intensity of mechanical or chemical effect as to produce actual lesions or disintegration of the protoplasts, as in wounds, corrosive chemical action, or desiccation, electrocution, etc.

4. External Forces to which Protoplasm Reacts. The principal forces to which living matter responds in the methods described above are :—shock, contact, pressure, traction, chemical action,

moisture, gravitation, temperature, electricity, electro-magnetism, light, X-rays, and other manifestations of radiant energy. The irritable influence of any of these forces depends upon variations in their intensity rather than upon the actual intensity which they exert upon their organism.

5. Reaction of Organisms to Internal Forces. The activity of any group of substances, or of any organ in the protoplast may set free forces which act as stimuli in setting up irritable reactions in other parts of the protoplast, or organism. The molecular motion set up by such stimuli may traverse long distances and incite reactions in portions of the body distant from the place of origin. It is this mechanism which correlates the activities of the entire body of the plant and gives it an automatic control over the functions of all of its organs (See correlations).

The character of the internal stimuli, and the method of transmission of their molecular effects is most imperfectly understood. Manifestations of such automatism are most plainly apparent in the behavior of growing points, diverse carpotropic phenomena, and the general axial arrangement and development of the members of the body. Thus the transferrence of food-material from one part of the body to another, the deposition of reserve matter, the activity of buds, the formation of enzymes, the division and behavior of embryonic tissues are not explainable by reference to the simple chemical and physical activities of living matter, but are controlled by its self-regulatory mechanisms.

6. Tonicity. The principal forces necessary for the continued activity of living matter, which may be designated as trophic factors, are moisture, food, and various forms of radiant energy. Protoplasm is in a condition for the normal performance of its functions only when these forces act upon it with a degree of intensity to which its accumulated experiences have accustomed it. Thus a cell carries on its entire group of vegetative functions only at certain temperatures, in which it is said to be in a state of *thermotonus.*

7. Critical Points in the Action of External Forces.   Within the tonic range of any force there is a degree of intensity at which the organism carries on the functions, most directly affected by this force, the most rapidly and to the greatest amount.   This point is the *optimum* of the agent in question.   If the intensity of the agent is increased a point is reached, the *maximum*, where the functions concerned are inhibited.   If the intensity is decreased from the optimum, a point is reached where the functions cease, and the *minimum* is determined.   These critical points are by no means identical in regard to different organisms, or the different stages of the same individual, and vary with the complex of all of the incident forces.

8. Rigor.   A decrease of the intensity below the minimum or an increase above the maximum, exercises diverse effects upon living matter.   In some instances mechanical injury is produced, in other instances disintegrating chemical action ensues, or in response to some forces the protoplasm becomes rigid and unresponsive at the unfavorable conditions.   Death follows the undue and rapid increase or decrease of most of the trophic factors.   The attainment of less favorable conditions of intensity by sudden changes within the tonic range, also brings about a *rigor* in which the organism is unresponsive to stimuli.   The rapid repetition of changes in an incident force backward and forward over a given range of tonic intensity may induce a state of *tetanus*, or rigid inactivity.

9. Irritability.   Sudden variations of the intensity of an incident force may induce changes in the activity of the organism greatly disproportionate to the amount of change in the incident force (3).   Generally such responses ensue only when the organism is in a state of tonicity to the force in question if it is a trophic one. The amount of variation of any given force acting upon a plant necessary to produce a response or constitute a stimulus varies geometrically with the amount of the force acting upon the organism at the time the change is made.   Furthermore the amount or amplitude of the response varies with the total amount of the stimulating force (Weber's law).

10. Reactions may be Morphologic or Physiologic. The reactions which follow the reception of any stimulus may occur immediately, within a few seconds, or may be delayed for hours or even days. These reactions may consist in the alteration of the intensity, rapidity and direction of growth ; of the intensity and character of the metabolic processes ; of the rate, rapidity and method of reproduction, alteration of the position of the body by flexions or locomotive action ; of the character and extent of the nuclear and cell cleavages, and of the formation of new tissues, entailing changes in form and mechanical relations to environment.

11. Motile Reactions. Motile reactions are most easily apprehended and estimated, and the organization of irritability reaches its highest visible development in the tissues devoted or concerned in the movements and orientations of the body ; it will be most profitable therefore, to begin the study of the relation of the plant to incident forces by a consideration of this form of activity. Such procedure is of still further advantage, because suitable material for the experimental demonstration of these phenomena is most easily procurable in the autumn or the opening of the collegiate year.

12. Mechanism of Irrito-motility. Two general forms of mechanisms for the performance of movement and other manifestations may be mentioned, which differ chiefly in complexity. One has been developed to the greatest extent in the animal kingdom, while the other alone is exhibited by plants. The first receives the stimulus in sensory organs, communicates some kind of molecular motion to a central organ of the nervous system where it may come to consciousness, and is complicated with psychical processes before it traverses a second series of conductors to the organs exhibiting the phenomena of reaction. The other, exhibited by plants, receives the stimulus in sensory regions which may or may not be differentiated morphologically, and in which the stimulus gives rise to a second kind of molecular motion which is transmitted more or less directly to the organ

or region which is designed to make the adjustments in response
to such stimuli.   The irritable system of the plant may be said to
be *reflective*.

FIG. I. Diagrammatic representation of the course of the *fibrillæ* supposed to form the path of transmission in the plerome of roots of *Allium cepa*. The thickness of the walls has been exaggerated to bring out the interprotoplastic threads. After Němec.

13. Sensory Organs and Zones.   Radiant forces may penetrate the body of the plant easily and reach internal cells almost as readily as external ones.   As a consequence of this fact no plants are known which have developed special organs or cells for the reception of stimuli of this character and of gravitation, although the last named force is supposed to act as a stimulus only upon certain embryonic cells in the tips of roots while certain similar specializations of phototropic action are shown.   The reception of chemical and mechanical stimuli however, can be accomplished only by peripheral protoplasts, and in some species in which instant perception of the stimulus and rapid reaction are of advantage the sensory cells and the motor mechanisms are highly developed with great morphological differentiation.   This is to be seen in the tentacular formations on the leaves of *Drosera*, and the epidermal cells of tendrils.   Furthermore the cytoplasmic layer of the cell is probably the functional organ in such action since its position is undisturbed by developmental changes.

14. Transmission of Impulses.   The action of a stimulating force upon the sensory elements may give rise to a new molecular motion the effects of which

are capable of transmission to neighboring protoplasts, or to distant regions of the body. It has been supposed that the disturbances thus communicated might consist of variations in electric potential, diosmotic changes, physical vibrations or hydrostatic changes in pressure, although it is probable that the impulse is in itself a characteristic phenomenon of living matter and not directly assignable to any of these comparatively crude classifications of phenomena; it is furthermore not proven and not necessarily true that impulses in all plants are identical in character.

The path by which impulses travel has not been identified in any single instance. It is found, however, that transmission is effected more readily in some directions than in others, and that the line of readiest transmission agrees with the location of certain fibrillar structures in cells and with the well-known interprotoplastic threads of cytoplasm. The fibrillæ which serve in this supposed transmission are specialized only in their arrangement and do not offer a parallel to the nervous tracts of the higher animals.[1]

[1] Němec, B. Die Reizleitung und die Reizleitenden Strukturen bei den Pflanzen. Jena. 1901.

II. RELATIONS OF PLANTS TO MECHANICAL FORCES

15. Mechanical Shock. Mechanical shock in its various forms is a kind of stimulation to which protoplasm has been subject continuously since its existence began, and it has developed the power of a number of adaptive morphological responses of which the alterations in stems and other structures as a reaction to strains and stresses may be taken as an example. Of the directive and metabolic responses to this class of stimulation but few have a definite economic purpose. The contractile movements of plasmodial forms, the movements of certain organs in carnivorous species, of pollinating mechanisms, and of tendrils are of this number. On the other hand, a large number of plants exhibit marked reactions to shock in the form of movements, metabolic variations and exchanges with the surrounding medium which the most thorough investigation has failed to invest with a purpose. New relations of the plant may be discovered however, which will interpret these reactions. Among the responses of the plant to shock of unknown purpose are the movements of "sensitive" plants, the increase in transpiration and the behavior of stomata.

Reactions to mechanical stimuli offer well-marked demonstrations of the relations between the amount of the stimulus and response, since the energy of the stimulus may be easily measured and the amplitude of the response estimated. The sensory and motor mechanisms involved are also usually highly differentiated, making them most profitable objects for the introduction to the study of irrito-motility.

16. Contractile Reactions to Shock. Collect fruiting forms of some myxomycete such as *Trichia, Arcyria, Stemonitis* or *Didymium* and sow the spores on a piece of the decaying wood or other

substance on which the organisms were found, under a small bell-
jar or moist chamber at a temperature of 20 to 25° C. After the
spores have germinated and the myxamoeba have attained a size
convenient for manipulation, which will need a few days, mount a
few in a drop of water on an ordinary microscopic slide at room
temperatures. The amoeba secured from a pool of stagnant water
or aquarium containing decaying leaves will serve equally well.
After the organisms have regained their normal condition and are
slowly moving in the field of view tap the cover-glass smartly
with a pencil. Note the retraction of the pseudopodia or irregular
extensions of the body and the contraction of the entire mass to
a more or less rounded form. Note the length of time before the
pseudopodia are again extended. Repeat a number of times.
Note appearance and behavior of nucleus and vacuoles.

The demonstration may be accomplished with almost any
plasmodial organism and is doubtless a protective movement for re-
ducing the surface to a minimum and thus lessening the liability
to injury. The reactions to chemical stimuli of an injurious char-
acter are generally similar.

**17. Influence of Mechanical Shock upon the Streaming Move-
ments of Vegetative Cells.** Mount a sound leaf of *Philotria*
(*Elodea*), stamen hairs of *Tradescantia*, hairs from the epidermis
of *Cucurbita*, or *Cypripedium*, in water at room temperature, and
observing streaming movements of protoplasm. Having secured
a good view in which the moving strands are to be seen clearly
with a magnification of 250 to 300 diameters, rack up the objec-
tive slightly, and tap on the cover-glass smartly with a pencil.
A heavier shock will be necessary to secure a reaction than was
used in the previous experiment, because of the outer protective
walls surrounding the protoplasm. Focus again on the same
cell as before and note the change in the movements, and the
consistency of the protoplasm with respect to its granularity.
Allow the preparation to remain on the stage of the microscope
for half an hour and examine at intervals of five minutes. Note
the period of recovery and the resumption of movement. Com-

FIG. 2. Successive positions of *Mimosa* after stimulus has been applied to tip of leaf. *A*, position a few seconds after stimulus has been applied at *f*. *B*, after impulse has reached the base of the leaf. *C*, after the effect of the stimulus has been transmitted to the entire body of the specimen and is traversing one leaf from base to apex.

pare reactions in different species. Crush a few cells and note appearance of protoplasm.

The shock given the material in mounting it on the slide, and the contact with water may stop the movement, so that it is often necessary to wait a few minutes for its resumption. It is important to keep in mind the fact that the cells of the organs affected in the following experiments undergo similar changes in response to shock, although all living cells do not show such distinct moving strands.

18. Motile Reactions of a Higher Plant to Mechanical Shock. Provide a well-grown specimen of *Mimosa pudica*, or any of the nearly related, and similarly irritable species, and place it in the greenhouse or experimental chamber where it will be kept at a temperature of 25 to 30° C. in a moist atmosphere, and the soil well supplied with water. After a period of complete rest of a day, in which the specimen has not been jarred or jostled, strike a quick sharp blow on the tip of one of the expanded pinnules with a pencil, or give it a snip with a pair of forceps. Note the immediate change in position of the parts actually struck, the successive closure of the pairs of pinnules toward the base of the leaflet, the following slight movement of the leaflets, and the change in the angle

of the petiole with the main stem. The angles should be meas-
ured exactly with a protractor. The influence of the stimulus
may be conducted up or down the stem to other leaves in which
it will be transmitted from the bases toward the apices, causing
the movement of the petiole first and of the leaflets last.

The demonstration of reac-
tion to shock may also be
made with *Biophytum sensiti-*
vum. In this plant the leaves
are simply pinnate. A stimu-
lus applied to the terminal pair
of leaflets is transmitted the
length of the rachis only, and
does not pass into the other
leaves attached to the crown,
ordinarily, although Haber-
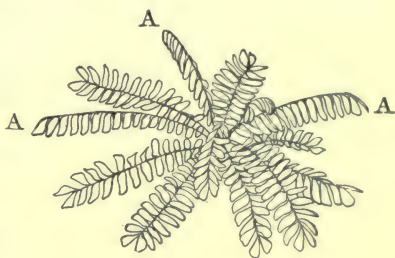
FIG. 3. *Biophytum sensitivum. A,*
A, A, leaflets after mechanical stimulation.

landt[1] demonstrated transmission through stems and flower
stalks. A notable feature of the reaction in this plant is the fact
that in response to a single stimulation the leaflets close toward
each other in pairs through a small arc, and then after a short
interval make a second movement in the same direction.

19. Rate of Transmission of Impulses or Stimulus-effects. With
watch in hand snip the terminal pair of pinnules of a normal
leaf of *Mimosa* by means of a pair of scissors or forceps, and
note the number of seconds elapsing before each pair of pin-
nules closes together as the impulse traverses the midrib, and be-
fore the whole leaf falls down by the action of the main pulvinus
at its base. Next note the time elapsing before the impulse
reaches the leaves above and below the one originally stimulated.
Measure the distance from the point at which the stimulus was
applied to every point of action and estimate the rate of trans-
mission in the different organs. If the stimulus applied does not

[1] Ueber die Reizbewegungen und die Fortpflanzung bie *Biophytum.* Ann. Jard.
Bot. d. Buitenzorg. Second Supplement, p. 33. 1898. See also MacDougal.
Transmission of impulses in *Biophytum.* Bot. Centralb. **77: 297.** 1899.

affect the other leaves by transmission, use a burning match or heated rod instead of the forceps to irritate the pinnule at the beginning of the experiment. Make similar measurements with *Biophytum*. Repeat both experiments and from the data thus secured make out the average rate of transmission. Does the impulse travel at the same rate in the direction of the root and toward the apex of the shoot? The time elapsing between the reception of the stimulus, and the reaction includes also the period necessary for the stimulus to be converted into a different kind of molecular motion which traverses the tissues and sets free the specific energy of the reacting mechanism. The impulse will be found to travel at the rate of 8–20 mm. per second in *Mimosa*, and 1–3 mm. per second in *Biophytum*.

20. The Structure and Action of the Motor Organs. Cut transverse and longitudinal sections of the pulvini at the bases of the petioles and petiolules in *Mimosa* and *Biophytum* and examine their structure with magnification of 400 or 600 diameters. The chief features will be found to be a central cord of fibrovascular tissue, encased with a collenchymatous sheath. Outside of this is a cylindrical mass of highly turgid parenchymatous tissue, which is under such tension that the sections curl when placed on the glass slip for examination. The communication of the impulse to the pulvinus probably causes a contraction of the protoplasm of the cortical cells of the lower side of the pulvinus similar to that exhibited by the amoeba (16) and allows some of the water in the cell to pass out into the intercellular spaces. This reduces the size of the cells concerned, and shortens that side of the pulvinus, thus causing a movement. The central strand of the pulvinus behaves like a thin rod of flexible steel sheathed in gutta percha.

In *Mimosa* the shortening of the lower side of the pulvinus allows the leaf to drop in response to its own weight, in addition to the pressure of the opposite side of the organ. Fasten a plant in an inverted position and when the leaves are normally expanded, apply a stimulus to the tips of a leaflet and compare the

resultant reaction with that obtained from a normal and upright specimen.

21. Recovery of Normal Position after Shock. Jar a suitably expanded specimen of *Mimosa* in such a manner that all of its leaves drop and its pinnules close. Measure the distance between the tips of the closed pinnules and mark position of tips of leaflets. Note exact length of time before the resumption of the original position begins. During this period the contractile cells are slowly regaining their former degree of turgidity by the reabsorption of the previously excreted water. Compare this period with that of the amoeba or streaming cells after shock.

22. Sensory Elements. Practically all of the epidermal cells of the shoot of *Mimosa*, except some parts of the inflorescence and the upper side of the

FIG. 4. Section of pulvinus of *Mimosa*. *a*, *b*, turgid parenchyma of upper and lower sides, *c*, bud. *d*, parenchyma of stem. *e*, pith. After Green.

pulvinus are capable of receiving the mechanical stimulus and converting it into an impulse which may be transmitted to distant parts of the body. Even the cotyledons are slightly "sensitive." When the pinnule is struck the effect is generally given direct to the small pulvinus at its base, but the cells of the lamina are capable of receiving the stimulus and transmitting its effects, as may be shown if the pinnule is gently pinched, or snipped, or touched with a small heated wire.

23. Method of Transmission of Impulses in Mimosa and Similar Plants. A system of long tube-like cells lying near the fibro-vascular bundles, and generally turgid are supposed to be the organs of conduction of impulses consisting of hydrostatic disturbances of the contained fluid. The exact demonstration of such transmission has not been made however. The extremely small size of the vessels, would render the gross movement of the water very difficult and slow on account of the enormous friction to be overcome. Artificial impulses given the smooth ends of several branches supporting normally expanded leaves by powerful pumps and endosmotic solutions failed to secure a reaction.[1] Furthermore impulses may be transmitted through a section of dead stem or petiole and cause a reaction as demonstrated by the author and others.[2] This is of sufficient interest to warrant its repetition. Select a small vigorous specimen of *Mimosa* and place it in a horizontal position. Wrap a section of the stem 2 cm. long with two or three thicknesses of cloth. Pour a steady stream of boiling water on this for five minutes. Repeat a second and third time at intervals of half an hour. Drive a small stake in the earth in the pot and secure the stem to it by means of suitable cords, and set in an upright position. Care must be exercised that no portion of the stem is injured beside the section touched by the boiling water and wrapped with cloth. Suspend a small vessel of water conveniently near, and run a strip of cloth from it to the bandage around the stem to prevent the treated section from drying out, and reducing its capacity for conduction of water to the leaves. After all of the leaves have regained the normal position and the plant has the proper temperature, give a harsh stimulus to the stem by cutting into the cortex with a razor or if leaves are to be found both above and below the killed section, hold a burning match to the tips of a leaflet. The stimulus-effect

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[1] MacDougal. Mechanism of movement and transmission of impulses in *Mimosa* and other sensitive plants. Bot. Gazette, 22: 293. 1896.

[2] Němec, B. Reizleitung und die Reizleitenden Strukturen bei den Pflanzen. Jena. 1901.

will be transmitted through the section of the stem which has been killed. After this has been demonstrated the treated portion should be examined by cutting sections and placing under the microscope. Impulses are thus seen to traverse dead tissues, and have been proven to pass through even desiccated portions.

24. Repetition of Stimuli. A single stimulus to produce a reaction must have a certain intensity determined by the organism, and any force when applied in a less degree does not call out the full response. The stimulating force does cause disturbances in the molecular motion of the protoplasm however, even when too weakly applied to produce a reaction. This disturbance endures a brief time and unless supplemented, its excitatory effect is lost. If however, one insufficient application of the force is followed by a second, or by a series before the influence of the preceding has been lost, the effects of the successive applications of the force may be added to each other and finally accomplish excitation. In this way a series of weak applications of a force may produce stimulation. If the strength of each of the series of applications is the to increased point where each alone would constitute a stimulus, the reaction resulting will be of greater amplitude than that resulting from a single stimulation. The continuation of the series of stimuli after the reaction has been shown will have the effect of holding the organ or organism affected in a contracted or reacted state known as *tetanus*. The tetanized condition is accompanied by an increased release of energy on the part of the organism, as if it were undergoing a number of successive reactions. After a time, however, which varies with the character of the stimulus and the organism, the release of energy undergoes a diminution and if the stimulus is not so strong as to throw the living matter into a state of rigor, the organism becomes accustomed to it, and even resumes its normal condition during the continuation of the stimulating force. This *accommodation* is most marked and necessary in the relations of the plant to radiant forces but it is also shown toward others, especially that of chemical action.

3

25. Summation of Impulses.   Secure a few good specimens of *Dionaea* growing in pots at a temperature of 25 to 30° C. Observe the mechanism of the curiously formed leaves.   Objects roughly placed on the upper surfaces of the lobes cause them to fold up together applying the inner (upper) sides together.   Now carefully touch one of the strong hair-like bodies growing up from each lobe of a plant hitherto undisturbed.   If a firm gentle blow be given with a thin splinter of wood in such manner that but one
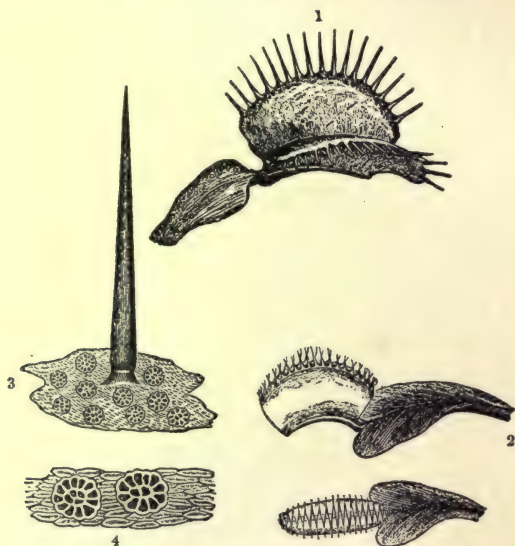
FIG. 5.   *Dionaea muscipula.*   1, open normal leaf.   2, closed.   3, irritable spines ($\times$ 50).   4, glands on surface of leaf ($\times$ 100).   After Green.

shock is given to the hair no movement will follow.   Repeat the blow on the same hair or any hair of the leaf within ten or fifteen seconds and the characteristic closure of the lamina will result. Give the blows with an interval of thirty seconds between and note result.   Give a succession of very light blows separated by twenty-five to forty seconds.   How many are necessary to secure movement ?[1]

[1] Dean, B. Dionaea. Its life habits and conditions. Trans. N. Y. Acad Sci. **12** : 9. 1892. See also, MacFarlane, J. M. Contributions to the history of Dionaea muscipula, Ellis. Cont. Bot. Lab. Univ. Penn. **1** : 7.   1892.

Strike the terminal leaflets of a normal specimen of *Biophytum* so lightly that no reaction ensues and repeat at intervals to determine the memory, or time during which the stimulus-effects are retained and cumulated, finally producing full excitation.

26. Reactions of Stamens of Opuntia to Shock. The flower of *Opuntia* contains a single central style surrounded by a number of shorter stamens. Bees or other insects entering the flower in quest of honey pass down the style, touching the filaments of the stamens. As a result of such stimulation the stamens curve inwardly toward the
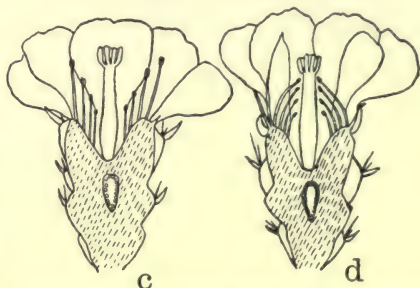
FIG. 6. Sections of two flowers of *Opuntia*. *c*, with stamens in normal position. *d*, with stamens bending toward the style in response to shock of rough object or insect. After Toumey.

style, forming an arched cage with the style in the center, in which the bee is imprisoned. In brushing aside the anthers to escape the bee carries away some of the pollen, which may be left in another flower, effecting cross pollination. The curvature of the filaments is so rigid that the bee may be held for several minutes, and its struggles act as repeated stimuli, causing tetanus of the filaments. After a time, however, the organs become accustomed to the repetition of the stimuli, the tetanus is relaxed and the filaments straighten out to their original position, allowing the insect to escape. If it is not possible to observe this action, imitate the behavior of the bee with a small wire or wooden splinter.[1]

FIG. 7. Pistil of *Mimulus*. *b*, expanded position. *a*, after irritation of the stigmatic surfaces. After Belzung.

Similarly sensitive stamens may be found in *Mammillaria*, *Echinocactus*, *Echinocereus* and other reactions may be seen in *Berberis*, and in some of the Cichoria-

[1] Toumey, J. W. Sensitive stamens in the genus *Opuntia*. Asa Gray Bull. 7 : 35. 1899.

ceae.   Portions of the style or stigma are sensitive in *Martynia*, and *Mimulus*.[1]

27. Accommodation of Mimosa to Repeated Mechanical Shock. Place a well-grown specimen upon a drained bench in a greenhouse in strong light and a temperature of 25° to 30° C.   Fasten a sheet of rubber cloth around the base of the stem in such manner that water falling upon the plant will not reach the soil in the pot.   Set a tub or cask on a support a meter or two above the plant and arrange a connection with a water system that will keep it full.   The water in the cask will thus acquire the approximate temperature of the air.   Arrange a siphon tube with a spray nozzle so that a constant spray will fall on the leaves of the plant in such manner as to resemble a shower of rain.   If a system of warm water is at hand the spray may be given directly from the pipes, but care must be taken to secure the proper temperature as above.   Note the behavior of the leaves when the shower begins.   Observe the plant at intervals throughout the day, and determine the time necessary for it to emerge from the state of tetanus into which it is thrown by the repeated stimulation of the falling drops of water.   On the following day, after the leaves

Fig. 8. Nozzle suitable for spraying *Mimosa*.

have resumed their accustomed position and have become accustomed to the repetition of the stimulus, send a sudden gust of air against the leaves.   This mechanical stimulus of a different intensity and direction will cause closure of the pinnules and other

[1] Hansgirg, A.   Ueber die Verbreitung der Reizbaren Staubfäden und Narben, sowie der sich periodisch oder blos einmal öffenden und schliessenden Blüten. Bot. Centralblatt. 43 : 409.   1890.

reactions. Strike an expanded leaf and note the result. Shield a small flame or heated wire and touch the tip of a leaflet and observe the reaction. Snip the tip of another leaf with a pair of forceps or cut away a portion with a pair of scissors. The reaction is again shown.

Place the second specimen where it will receive a strong stimulating current of air and carry through a series of tests similar to the above. A device consisting of a centrifuge with a small pliant rod such as a strip of bamboo attached to one arm may also be set up by which the stem may be given delicate and repeated blows, and the behavior of the plant followed through a parallel course of reactions.

28. Influence of Shock upon Metabolic and other Processes. The effect of any stimulus is to set up new or additional molecular movement in the protoplasm. These movements may result in external movement as in previous experiments, entailing the release of energy and the increase of the metabolic processes concerned. Still further the influence of the stimulus may increase the respiration and other processes in addition to the specific energy release. Shock is followed by the appreciable increase of the excretion of carbon dioxide, the decrease of surplus foods in the cell, and by an increase in the amount of water thrown off in the transpiratory processes. The last named result is probably due to the greater amount of water present or thrown into the intercellular spaces by the contractile action of the cells.

29. Effect of Shock upon Transpiration. Select a vigorously growing specimen of a tomato, geranium, or some leafy twig, and fasten it to a potometer (See transpiration and potometer). An hour or two later after the rate of transpiration is fairly steady note the rate at which water is taken up by the shoot. Arrange a centrifuge in such position that a very thin strip of bamboo attached to one arm will strike the stem as the apparatus revolves. Run the centrifuge to give the stem a series of shocks for a period of fifteen minutes, that will not injure the plant mechanically. Now take readings on the potometer for the next fifteen minutes.

If no machine is at hand, imitate its action by a succession of blows with a small wooden rod or pencil. Determine the length of time before the effect of the shock on transpiration can be seen, and the actual amount of such acceleration.

This experiment may also be carried out by weighing the potted plant during an hour of quiet and also during a second hour after the shock has been given, although the actual amount of difference in the water thrown off may not be easily appreciable by this method.

30. Contact as Stimulus. Another form of mechanical stimulus which differs from shock in degree rather than kind is that of contact. Two distinct phases of the influence of contact upon the plant are observable. One form which may be designated as *thigmotropism* is exhibited by some roots and the special organs of climbing plants. The sensory elements in such plants may perceive a stimulus made by the weight of a body of a weight of not more than a fraction of a milligram lying in contact with them. It is possible to regard contact stimulation as an instance of summation of an immense number of shock effects, from projections so minute that their separate impact would not be perceptible. Again it is to be said that the perception of contact as a stimulus is developed only in certain specialized forms, in which this form of irritability is of special use in relation to environmental factors.

31. Reactions to Contact. Secure specimens of *Sicyos*, *Micrampelis*, *Cucurbita*, or *Passiflora* growing at a temperature of 25 to 30° C., and select nearly mature tendrils. Make a careful drawing of the profile of the organ and then touch the surface which is slightly concave with a rod of wood or iron. With watch in hand note the number of seconds or minutes elapsing before a curvature ensues. This would range from a second or two in *Passiflora* to two hours in *Vitis*. Follow the course of the reaction, making drawings of the profile of the tendril every fifteen minutes. Note the length of time during which the contraction endures, the resting period, and the length of time necessary for the resumption of the original unstimulated position.

32. Determination of the Character of the Bodies which may Act as Contact Stimuli. Dip a glass rod in liquid gelatine, and after it has solidified so that it will not drop from the rod, touch the sensitive surface of the tendril with it. Does a reaction follow? Set the rod aside until the gelatine hardens and presents a rough outer surface. Repeat the experiment, and note result. The occurrence of a response to the contact of a tendril with a body would appear to depend upon the smoothness of the surface, or rather the size of the minute projections which press against the convex outer surfaces of the receiving surface. Soften the dried gelatine in water and dip in fine sand. Touch the tendril again, and observe results.

33. Transmission of Impulses in Tendrils. Fasten a small thread taut between the arms of a pair of calipers, moisten in India ink and touch the sensitive surface of a tendril at one point which will be marked with a coating of ink. Note the limits of the region in which curvature ensues. To what extent is this stimulus-effect transmitted?

34. Tetanized Condition of a Tendril. Fasten a cord or wooden rod in such position that the tip of a tendril will come in contact with it, and coil around it. Remove the cord or rod two hours after the beginning of the experiment. Repeat, allowing the tendril to clasp the support for three, four or five hours. How long may the tendril remain in a contracted condition and return to the normal? All of the curvatures are fixed after a time by growth-alterations in the cells of the tendril so that it is unable to uncoil or relax as did the leaflets of *Mimosa* or the stamens of *Opuntia*. The fixation of the curves of the tendril in this manner is a secondary phenomenon which enables the slender climbing plant to fasten its body permanently to a support. The pressure on the inner surface of the tendril acts as a second stimulus upon the growth processes, causing an exaggerated elongation of the convex side of the organ and decreased extension of the concave flank.

35. Localization of the Perceptive Zone. Tendrils which are flattened or show a bilateral structure are not sensitive on both

sides, or equally sensitive over all of the perceptive zone.   Make
a number of tests to determine the sensitive surface of tendrils of
the species named in the above experiment.   If possible compare
with some tendril of radial structure.   Make a second series of
tests to determine the relative delicacy of sensitiveness of the dif-
ferent parts of the perceptive zone.   Variations among different
species will be found.

36. Summation of Stimulus-effects.   Touch the irritable surface
of a tendril with a wooden rod for two or three seconds.   Repeat
with a second tendril which should
be irritated a second time after an
interval of five seconds.   Note the
difference in the amplitude of the
resulting curvatures.   The uncer-
tainty of giving the stimuli of equal
strength is such that the experi-
ment should be repeated two or
three times with different sets of
tendrils.

**37. Measurement of Force of
Contraction.**   Allow a tip of a vine
of the Passion-flower (*Passiflora*)
to rise above the top of a shelf or
table and fasten it firmly to an up-
right post by a cord tied around the
stem at the base of a tendril in a

FIG. 9.   Shoots of *Smilax* showing
tendrils.

proper condition of irritability.   Ir-
ritate the tip of the tendril until it
has clasped the arm of a spring dynamometer.   Then fix the
dynamometer firmly in an upright position at such distance from
the stem that the tendril will be extended its full length. After the
contraction due to handling has been lost, again adjust the dyna-
mometer so that the tendril is extended its full length.   Now rub
the sensitive surface with a pencil and note the contractile force
exerted upon the dynamometer.   A stress of .5 gram is quickly set

up. Allow the preparation to remain in place, readjusting daily
to take up growth in length of organ if it is not mature. The
portion of the tendril between the base and the point at which
it is fixed to the instrument will be thrown into coils exerting a
pull of 15 to 30 g. on the dynamometer.

FIG. 10. Dynamometer attached to *Passiflora* to measure contractile force and
also strength exerted in the formation of the free coils. *N*, support. *L*, fixed arm of
dynamometer. *B*, hinged arm. *E*, hook for attachment. *S*, spring. *F*, scale.
D, portion of scale reading amount of tension.

38. Structure of a Tendril. Cut out the most highly sensi-
tive portion of a tendril of *Passiflora* or other convenient species
and kill quickly in acetic alcohol. Imbed by the usual methods
in paraffin and cut longitudinal sections. Stain with differential
colors. Compare the structure of the same kinds of tissue in the

upper and lower sides.   Examine the epidermal cells of the con-
cave surface constituting the perceptive zone (Fig. 11).[1]
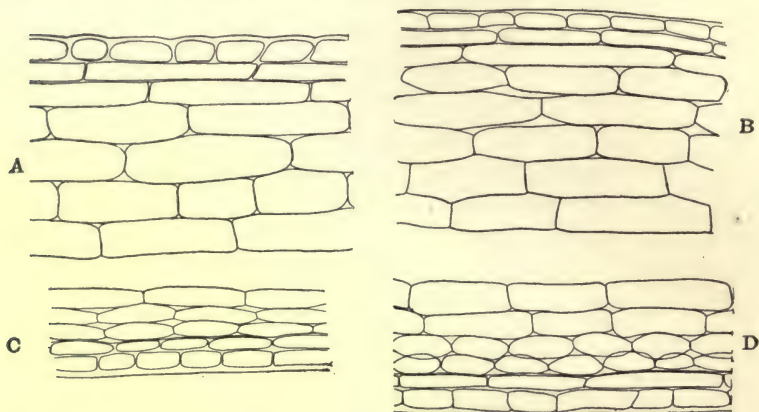
Fig. 11. Sections showing the character of the cell contours in a longitudinal sec-
tion of a tendril. *A*, epidermis and cortex of convex side before curvature. *B*, after
curvature. *C*, concave side before curvature. *D*, after curvature.

39. Comparison of the Irritability of Tendrils and Mimosa.
Allow a stream of water to fall in a shower upon sensitive tendrils
as in the experiment with *Mimosa* (27).   Does curvature follow?
Arrange to have the water mixed with a quantity of fine sand.
This can be done by placing a quantity of sand in the bottom of
the vessel containing the water and stirring as the water flows out
through the siphon tube.   Note the result of the action of the
minute mineral particles.   Strike the stem at the base of an active
tendril so that it will be shaken violently.   Note the reactions to
shock.   Are they similar to those of contact?   Apply a steady
but gentle pressure to the tip of a leaflet of *Mimosa* taking care
not to crush or bruise the tissues.   Is *Mimosa* sensitive to contact?

40. Contact Reactions of Drosera.   Specimens of *Drosera*
should be cultivated in shallow wooden or earthenware dishes con-
taining peat and brought into a room kept at 20 to 25° C. for the

[1] MacDougal. Mechanism of the curvature of tendrils. Annals of Botany, 10:
373. 1896.

tests.   Place pieces of rotten wood, boiled meat, or boiled egg, or bits of glass no larger than a pin head on the tips of the glands of tentacles at the margin of the leaves.   Observe the tentacles with a lens and note the latent period before movement is observed, the period of curvature, and the final position of the stimulated organ.   Does relaxation of the movement occur while the object remains on the tentacle ?   Do all of the objects mentioned secure equal reactions ? [1]
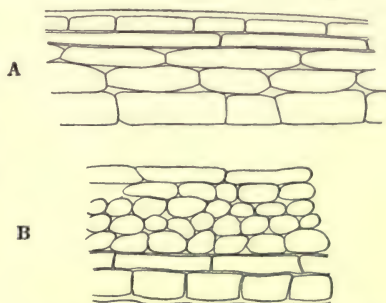
Fig. 12.   *A*, tissues of convex flank of tendril.   *B*, of concave flank after forming loose coils.

41. Contact Reactions of Tendrils of Ampelopsis.   The branched tendrils of *Ampelopsis* do not generally coil around supports. These organs are fastened to solid objects by the tips which undergo peculiar metamorphoses when they come in contact with them.   It is difficult to determine whether the resultant reaction is due to a contact sufficiently strong to be called a pressure reaction or not, but as has been noted the difference is not one of quality, so that it may be taken up here.   Examine the manner in which an *Ampelopsis* is fastened to a wall.   The tips are found to be glued to the support, and are enlarged to form large balls of tissue.   If an unattached tendril is examined with the microscope the transverse section will show a pith relatively large, a circle of fibrovascular tissue, with large medullary rays extending from the pith to the cortex.   A layer of collenchyma is present in the subepidermal layers of the cortex.   Select young tendrils or those recently fastened to a support and make out the changes undergone by the tissues in forming the attachment. [2]

[1] For changes in the tentacles during reaction see Huie, Quarterly Journal of Microscopical Science, 39: —.   1896.

[2] Lengerkin, A. v.   Die Bildung der Haftballen an den Ranken einiger Arten der Gattung Ampelopsis, 43: 337, 353, 369, 385, 401.   Bot. Zeitung.   1885.

42. Curvatures of Roots away from Solid Objects. Soak a number of peas or beans for a day in water and then place in moist chamber. When the roots are 2 cm. in length provide a large bottle with a wide mouth and a cork stopper. Fasten the seedlings to the under side of the stopper in such position that the tips of the roots will be directed perpendicularly, when the stopper is put in place. To attach the seedlings to the stopper bore a hole in a small cork of sufficient size to enclose the main root,
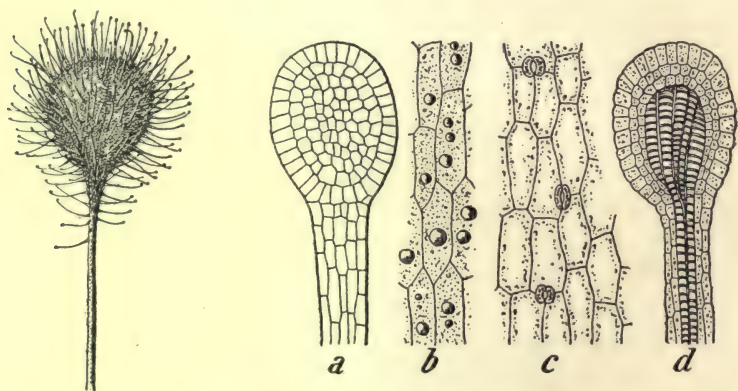
FIG. 13. Leaf of *Drosera*. *a*, surface view of tentacle. *b*, parenchyma cells. *c*, epidermal cells from base of peduncle showing stomata. *d*, section of tentacle. (From alcoholic material. After Belzung.)

holding it firmly, split it and place it around the root, fastening the halves together by means of pins driven through them. The cork may now be pinned to the stopper. Pour a few cc. of water into the bottle, and place in a room at 16° C. Cut squares of cardboard or paper 1.5 by 1.5 mm. and attach to the slope of the root-apex of half of the roots. These bits of paper should be fastened to the root by being moistened with gum and water, or shellac, in a position at right angles to the plane of the coty-ledons. Note the resulting curvatures of the roots during two or three days and compare with the form of the ones which have not been treated.

Set up the experiment as before but do not fasten objects to the

roots.   Pour a few cc. of water into the bottle and then fill it up
with fragments of rock or glass until the tips of the roots are
nearly in contact with them when the stopper is put in position.
Observe the behavior of the tips when they come in contact with
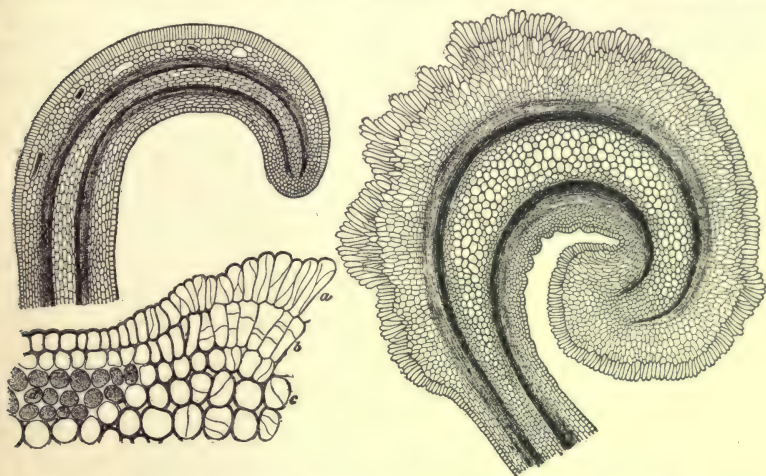
FIG. 14.   Showing section of tip of young tendril of *Ampelopsis muralis*, old ten-
dril of *A. quinquefolia* and partial cross-section of latter.   *a*, epidermis (dividing).
b, sub-epidermis (dividing).   *c*, cortical parenchyma (dividing).   *d*, collenchyma.
After von Lengerkin.

the objects in the bottle.   Both demonstrations will arrive at more
decisive results if the preparation is kept in a dark chamber.   This
negative reaction of curvature by the root in response to the con-
tact of a solid body is one which would prevent the tip from being
forced against any solid body in the soil in such manner as to injure
the growing point.

43. Compression, Stretching, Twisting and Bending.   The action
of any mechanical force which tends to change the form of a pro-
toplast, or a tissue generally causes a response of morphologic
nature, or a rearrangement of the elements of the living cell.
The principal reactions consist in the determination of the direc-
tion of the walls formed in the division of cells which process
may be exaggerated or set up anew under the influence of pres-

sure, alterations in the rapidity of growth, and the differentiation of the tissues. The reactions may really go so far as to determine the development or suppression of organs, as illustrated by the growth of secondary roots from the convex surface of main roots alone.

Mechanical stimuli of the above character may act from without, although the greater number originate within the body of the plant. Any organism consisting of more than one cell, has the development of its tissues or cell-masses affected by their mutual mechanical relations. In large plants such as trees, in which the living tissue is held firmly by a cylinder of dead wood and enclosed by a sheath of corky tissue the compression and its effects are most marked. The increase in weight of a fruit will cause morphological changes in the stem supporting it, and the multitudinous stresses set up by the stretching, bending and compressing action of neighboring tissues of unequal growth find responses in the tissues affected. The presence or absence of the action of some of these mechanical forces of external or internal origin may be accountable for the presence or absence of certain kinds of tissues in some plants. Thus, for instance, elongated bast fibres may be produced in certain plants by the action of external mechanical bending forces, although usually absent. The relative weight and density of the medium in which the organism lives are of course trophic conditions which may not be greatly varied without detriment to the normal processes of the organism.

44. Changes in Tendrils due to Pressure. The process of curvature in tendrils presses the inner flank against the object around which they are coiled, which induces altered development of the tissues, consisting in the increase of the wood ring, the tangential division of the hypodermal layers, with an increase in the thickness of the walls. Cut cross and longitudinal sections of a portion of a mature tendril which has been tightly coiled around a support for several days. Note the structure of the hypodermal layers, cortex, and fibrovascular ring. Compare

with the structure of a mature tendril which has not clasped a support.

45. Influence of Stretching Forces.   Compare the structure of the basal portion of a tendril which has been fastened to a support and borne the weight of a stem with the mature organ which had no stress of this character.

A most notable demonstration of the influence of such force may be made if one cucurbitaceous vine is allowed to trail along the ground which will support the weight of the fruits, while a second is trained to a trellis, and the petioles allowed to carry the weight of the large fruits.   This effect will extend to the portions of the vine affected by the weight.[1]

46. Differentiation of Embryonic Tissues under Compression. The influence of compression upon the development of tissues may be found by enclosing growing stems in rigid casts of plaster of Paris.   Select a vigorous specimen of *Vicia, Pisum* or *Phaseolus*, and a cork, which is about five times the diameter of the stem.   Bore a hole longitudinally through the cork and split the cork in halves and place the halves together, enclosing the stem in such manner as to fit it tightly.   Fasten the halves of the cork together in place by driving pins through them.   The cork should enclose the median portion of an internode.   Now curve a tough piece of manila paper around the cork and fasten it with pins in such manner that it forms a cylinder with the cork as the bottom.   Fasten the edges together with pins.   Fill a small evaporating dish half full of plaster of Paris and add water to it slowly, stirring carefully until a creamy paste is formed that can be poured easily.   Quickly fill the paper cylinder with the mixture and support it in the proper position until the cast hardens, which will need from 30 to 60 minutes.   Paper and cork may now be removed.   The rigidity of the cast will prevent increase in thickness of the stem and change the stresses among the tissues.   Ten days later cut away the cast by making two longitu-

[1] Pieters, A. J.   The influence of fruit-bearing on the development of mechanical tissues in some fruit trees.   Annals of Botany, **10** : **511**.   1896.

dinal channels down opposite sides, by means of a small saw, and
examine the tissues of the enclosed portion with those of an un-
treated part of the stem. [1]

**47. The Influence of Curvatures upon the Origin and Arrange-
ment of Secondary Roots.** Fill a root-cage consisting of a narrow
vessel with glass slides with sand or loose porous soil. Put a
number of germinating beans, or seeds of lupine on the surface of
the soil in such manner that the roots will pass down through the
soil near the glass sides. After the roots have attained a length
of 5 cm. adjust the root cage so that it will be tilted at an angle
of 45 degrees, and allow it to remain in this position until a fur-
ther growth of equal amount has been made, then tilt in the oppo-
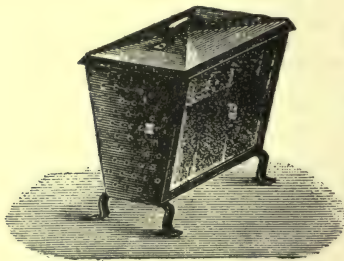
FIG. 15. Root-cage filled with sand
or sawdust.

site direction until the same elong-
ation has been made. Continue
this alternation of the position of
the cage until several curvatures
have been made, and numbers of
secondary roots have been formed.
The main roots will be found to
have an undulating outline as a
result of geotropic curvatures.
Note the position of the secondary
roots with respect to these cur-
vatures. These organs will be found to have arisen on the con-
vex surfaces of the roots alone. Determine the radius of curva-
ture of the main root. If a number of tests are made it will be
possible to determine the radius of curvatures necessary to pro-
duce this special arrangement of the secondary roots.

This special response of the root to the form in which it is
placed is due to the possession of a form of irritability which en-
ables the organism to control its external form or stature. The
suppression of the secondary roots on the concave side of the
main root and their accelerated development is not a direct re-

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[1] Newcombe, F. C. The regulatory formation of mechanical tissue. Bot. Ga-
zette, 20 : 441. 1895.

sponse to the tensions produced by such curvatures, as may be seen from tests made in which the tension of two sides of a straight root are thrown out of equilibrium.[1]   It is a direct reaction to forces which change the customary form of the organ.[2]

A   *B*
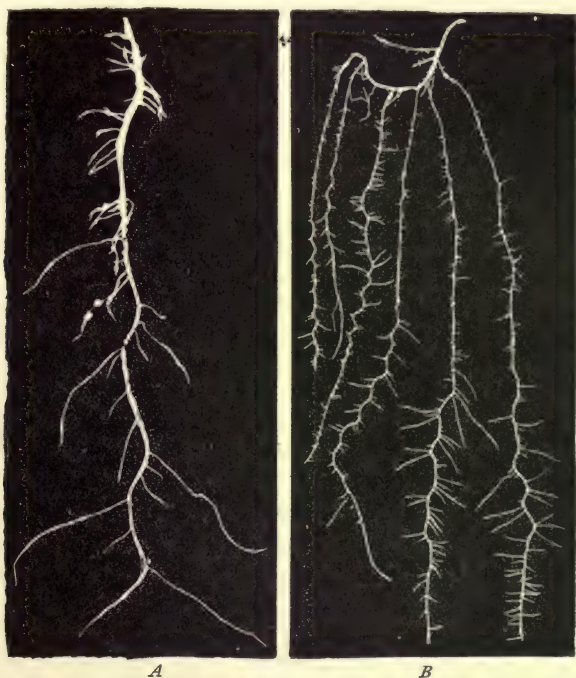
FIG. 16. Root-systems of *Pisum sativum :* *A*, developed in soil and curved geotropically. *B*, grown in water-culture, automatically curved.   Secondary roots originating from convexities.   After Noll.

48. Wounds, Lesions, and General Mechanical Injuries.   Intense mechanical forces which cut, tear, or crush the protoplasts or their membranes, exert a stimulating effect upon the neighboring uninjured elements as well as the entire organism in certain instances.   The most immediate result of such stimulation con-

[1] Noll, F.   Ueber den bestimmenden Einfluss von Wurzelkrümmungen auf der Enstehung und Anordnung der Seitenwurzeln.   Bonn, 1900.

[2] MacDougal.   Curvature of roots.   Bot. Gazette, **23** : 344.   1898.

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sists in increased respiration, as indicated by an increase in the amount of carbon dioxide exhaled, and the rise in temperature. Later reactions of a regulatory nature consist in the degeneration of the injured protoplasts, the renewed growth of neighboring resting cells, and some instances may be followed by the formation of a new tissue which has for its purpose the closure of the injured surface of the organism.   An example of this is to be

FIG. 17. Diagrams showing proper location of incision stimuli.

found in the callus formations of cuttings and trees.   A few organs such as roots and tendrils are capable of manifesting special movements in response to injury which may have for their purpose the withdrawal from the source of the injury.   It is to be noted in this connection that the destruction of a tissue will often be followed by purely mechanical curvatures, resultant from the release of a stress set up by the tissue when in its place. [1]

49. Traumatropic Curvatures of Roots.   Prepare a number of seedlings as in 42.   After the seedlings have been fastened to the cork cut the thinnest possible slice from one side of the tip, being careful not to remove more than one-fourth of the apical region, and not amputating the extreme tip (Fig. 17).   Put the cork in place with the roots in a perpendicular position and note results a day and two days later.   The seedlings may also be grown in moist sawdust in which they are placed in the same relative position after being treated as above.

50. Changes in Roots Stimulated Traumatropically.   Němec finds as a result of thorough investigations that the cells in the neighborhood of the injured surfaces of roots undergo various changes inclusive of a very marked vacuolization which it is be-

[1] Němec, B.   Die Fortpflanzung des Wundreizes.   Die Reizleitung und Reizleiten-den Strukturen bie den Pflanzen, 16.   1901.

lieved are connected with the transmission of the effects of the
stimulus.   Such effects may be observed by wounding roots by
incisions with a razor, and then fixing a half hour or hour later in
a mixture of picric, acetic and sulphuric acid in water.   The ob-
jects are stained *in toto* and sectioned by the usual imbedding
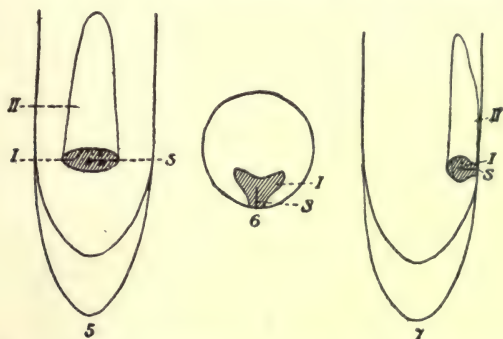
FIG. 18. Diagrammatic representation of the regions in which transmission of the
primary (*I*) and secondary (*II*) effects of wound-stimuli take place in the roots of
Allium cepa. Stimulus given at *S* and roots fixed 12 minutes later.   5 shows the re-
gion affected in the inner periblem.   6, the region affected in cross-section through the
point of incision.   7, the region affected as shown by a radial section through the
point of incision.   After Němec.

methods.   Transmission of the traumatic impulse takes place as
shown by these reactions most readily in the median and inner
periblem.

51. Movements of Mimosa in Response to Injury.   Secure a
few well-grown specimens of *Mimosa* and place them in a room
at 25° to 30° C.   Snip off the terminal pair of pinnules with
a sharp pair of scissors and note the reaction.   Touch the tips of
another leaflet with a lighted match or a heated rod.   Cut a slit
in the lower part of the stem of a specimen with expanded leaves
taking care not to jar the plant.   Compare the rate of transmis-
sion with that shown in response to shock.   Make a series of
wounds to determine whether injury stimuli are cumulative in
their effects.   Note the exudation of water from the wounds.

No definite purpose can be surmised for the reaction of *Mimosa*

to injury in this manner, and it is supposed that the wound acts simply in setting off or starting the machinery of irritability designed to give movements as reactions to other stimuli, a thing not unknown in other kinds of irritability.

52. Repeated Movement in Response to Injuries. Secure well-grown specimens of *Biophytum sensitivum* and snip away the terminal pair of leaflets with the scissors. The pairs remaining close in succession toward the base of the petiole or rachis. After a few minutes, when the leaflets have begun to recover from the contracted state of the pulvini and regain their former position, a second partial closure ensues, which also is in exact imitation of the normal reaction of the plant.

53. Traumatropic Curvatures of Tendrils. Select a number of active tendrils of *Passiflora* or any cucurbitaceous species, and snip off a section a centimeter in length from the tip with a single sweeping stroke of a sharp razor given on the non-sensitive surface. If a razor is not available use a pair of scissors though errors are introduced by the stimulus given the mechanism of ordinary curvature. Observe the wounded tendril, and note the peculiar curvature which is most marked in a region about 5 to 10 mm. from the end of the wounded organ.

54. Tissues Formed in Response to Injuries. The destruction of any of the living portion of a plant is followed by various regenerative processes, the most general of which consist in the formation of a layer of cork over the injured surface in herbaceous soft-bodied species and of callus in woody plants. Such production of cork is always due to embryonic tissues, usually the cambium, and the cork thus formed is a fixed tissue and not capable of further differentiation. Wounds in woody plants are healed or recovered by means of a special mass of embryonic tissues which may arise from any tissue except wood, hard bast and epidermal cells. Callus sometimes undergoes suberization of the walls and protects the wounded portion, or it may give rise to a phellogen forming an outer corky layer which serves this purpose. In the dicotyledons and gymnosperms the outer corky layer is differ-

entiated first while the callus is forming over the wound, and then later a cambium layer is developed from which wood and other tissues may be formed. The tissues formed from the callus do not form a complete union in most instances with the injured surfaces, hence the marks of old wounds may be found beneath the surface of the plant in the wood, especially. In the process of grafting in which a twig of one plant is fastened with its wounded surface in contact with the wounded surfaces of the stem on which it is to be grown, the two are welded together by the union of the callus which is formed by both. It is important that the tissues in the united elements should show a fairly similar structure and arrangement in order that grafting may be
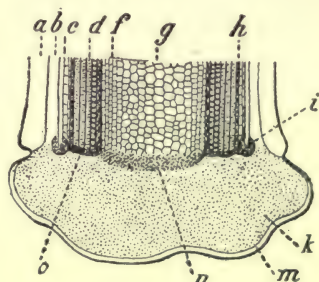
FIG. 19. Callus 32 days old on cutting of *Hibiscus reginae*. *a*, cortex. *b*, pericycle. *c*, bast. *d*, wood. *f*, xylem parenchyma. *g*, medulla. *h*, generative layer. *i*, *n*, *o*, cambium cushion on bast, medulla, and wood. *k*, parenchymatous tissue of callus. *m*, cork. After Belzung.

readily accomplished, hence the difficulty that arises in grafting between species of different genera, which however has been accomplished (See grafting).[1]

FIG. 20. Formation of cork in the outer region of callus of *Hibiscus reginae* (See Fig. 19). *a*, parenchymatous tissue. *b*, phellogen. *c*, cork. *d*, epidermal layers of callus. After Belzung.

55. Formation of Wound-cork and Callus. Make a number of cuttings of *Coleus* and *Begonia* and imbed in dishes of sand (See cuttings). Take one cutting one week later and make longitudinal sections through the portion ending in the cut surface. Make out the progress of the formation of a special tissue to protect the exposed part of the stem. Ex-

[1] Tittman, H. Physiologische Untersuchungen ueber Callusbildungen und Stecklingen holziger Gewächse. Jahrb. Wiss. Bot. 27: 164. 1895.

amine a second stem a week later and a third two weeks later.

Make a number of cuttings oɪ some woody plant, such as *Rosa, Salix, Hibiscus, Populus*, etc., and grow them in jars of water or sand.   Make an examination of the cut ends after two weeks, and at similar intervals for some time to observe the development of callus.   Cut off branches of vigorously growing woody shoots and note the formation of callus on the surface of the stumps.   Examine the structures resulting from the differentiation of the callus.[1]

[1] Sorauer-Weiss.   Treatment of the Shoot.   Popular Treatise on the Physiology of Plants.   134.   1895.

III. INFLUENCE OF CHEMICALS UPON PLANTS

56. General Chemical Relations of the Organism. An analysis of any mass of protoplasts reveals the invariable presence of twelve of the known elements, and of one or two others in certain organisms. Carbon, hydrogen, oxygen, and nitrogen are undoubtedly of the greatest importance to the cell so far as physiological performance and structural value may be estimated. The other elements may not actually enter into the plexus constituting the living substance, yet it is absolutely necessary that their compounds should saturate it in some form, and interlock in the special cases of metabolism in which each one is a minor, though necessary factor. The special part of each of the elements in the upbuilding and growth of the body will form the subject of a section on nutrition.

Organisms sustain tonic or trophic relations to the indispensable substances, and the three critical points may be distinguished in the varying intensities in which they may act upon living matter. In addition, the trophic or nutritive elements, as well as others to which protoplasm is totally foreign, exercise an irritable influence. The non-trophic elements need be present in a certain amount or intensity constituting the threshold of stimulation, in order to affect living matter in such manner as to secure a response, and a continuation of this intensity, unless the action is injurious will generally be followed by an acclimatization, or accommodation. Extensive increase of the intensity of a foreign element, or compound, may produce rigor and tetanus, and probably injuries resulting in disorganization and death.

The contact of a chemical compound may exercise a purely chemical effect by modifying the molecular motion of the substances within the circle of living matter, by satisfying, or setting free chemical affinities, thus modifying the performance of the

functions, or may bring about a rearrangement of the molecules or units of organization of living matter in such manner as to cause morphological changes, exaggerating or retarding development.

Then again the non-trophic compound may exert a purely physical effect upon the protoplasm, such as increasing or decreasing the amount of water content by changing osmotic pressure, or it may interfere with the exchange between the organism and the trophic factors of its environment.[1]   The intensity of the influence of all chemical substances upon the organism is conditioned to a great extent upon the temperature, concentration, structure, and pressure of the incident compounds.

57. Oxygen.   Oxygen is a constituent of the protoplasm, and as it is combined with other elements to form compounds which are more or less constantly excreted from the body, a constant supply of the free element is necessary for the existence of every organism.   This supply may be taken from the air as it is needed, from compounds in the medium or substratum, or it may be obtained from compounds absorbed and stored in the body of the protoplast.   The most important form of energy release in living matter is that which is brought about by the oxidation of certain compounds in the protoplast ; it is not definitely determined whether this oxidation concerns the material of which protoplasm is actually constructed, or merely the saturating and interlocking compounds.   The preponderance of evidence seems to favor the former view however (See respiration).

If the supply of oxygen is reduced, a correspondent diminution of the amount of energy released and convertible to various uses will ensue, and the performance of the organism lessened, except in anaërobic organisms (see above).   The continuation of only a partial supply of this element during a period of active growth and development will generally result in death.   During the resting period, however, such as that shown by seeds, long periods of total vacuity may be endured without deterioration.   On the

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[1] Livingstone, B. E.   On the nature of the stimulus which causes the change of form in polymorphic green algae.   Bot. Gazette, 30 : 289.   1901.

other hand an increase in the proportion of the oxygen does not accelerate the release of energy, or materially modify the performance of the organism except so far as it affects the pressure of the other atmospheric elements.

58. Streaming Movements of Protoplasm in the Absence of Oxygen. Mount a leaf of *Philotria*, *Nitella*, or a hair which shows a distinct streaming movement, on a cover-glass 2 cm. in diameter and invert over the opening of an Engelmann gas chamber. Seal the cover to the chamber by a small drop of oil smeared around the edges of the glass and connect the chamber with a hydrogen generator. A number of wash bottles containing a solution of potassium permanganate should be connected in the system between the generator and the chamber in order that all impurities should be taken out of the gas. Fasten the attention upon a well-defined strand in a cell and then open the stopcock to allow a current of the gas to flow through the chamber to replace the atmospheric air. After a few minutes, close the stopcock, cutting off the flow of gas, and then quickly clamp the free end of the tubes leading from the gas chamber. This will seal the speci-
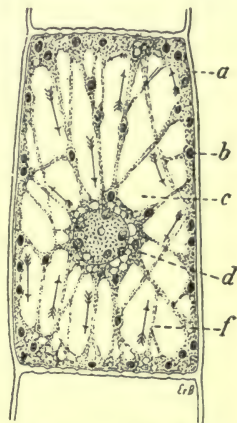
FIG. 21. Cell from a hair of the squash. *a*, wall. *b*, parietal layer of protoplasm with chloroplasts. *c*, vacuole. *d*, small vacuoles. *f*, strands of protoplasm in which moving plastids may be seen. After Belzung.

men in an atmosphere of pure hydrogen. Note the endurance of the movement. Calculate the rate of movement before beginning, by following the movement of a particular granule around the circuit of the cell. Repeat the observation after the hydrogen has been put into the chamber, noticing the change in the rate. After movement has ceased, disconnect the fittings and note the length of time necessary for the resumption of movement. Repeat the experiment with the same material, and compare data with that obtained from the first test.

Perform the experiment, using carbon dioxide from a suitable generator, and compare data with above. The exclusion of the oxygen may also be accomplished by mounting the objects in olive oil. Removal from the oil and washing in fresh water will be necessary to secure a resumption of the movement. It is to be kept in mind that rough handling will stop the movement in
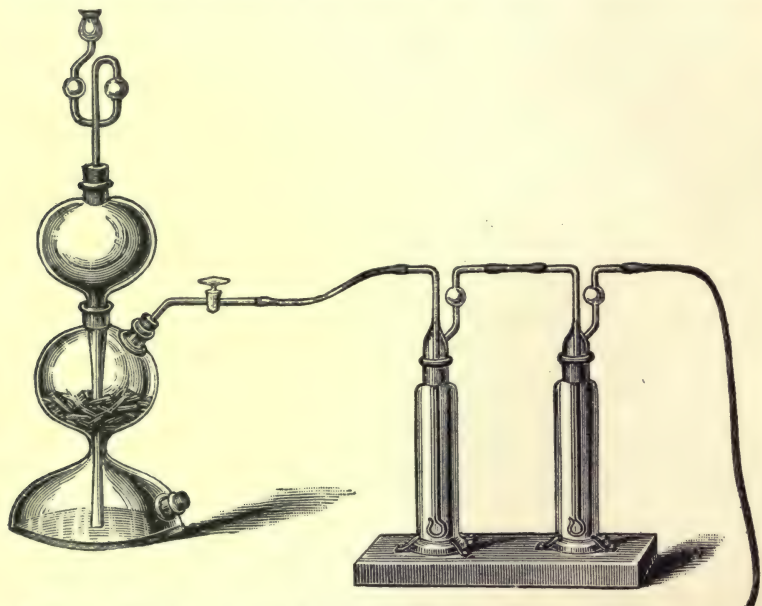
FIG. 22. Kipp's apparatus for production of hydrogen. The middle globe is partly filled with strips of zinc and a weak solution of hydrochloric or sulphuric acid is poured into the bent thistle tube until it rises into the chamber containing the zinc. The gas escapes through the outlet at the side and passes through a solution of potassium permanganate in the wash bottles. Closure of the stopcock in the outlet tube drives the acid back into the upper chamber and stops the evolution of gas.

consequence of the shock given. The organism is not subject to the influence of hydrogen under ordinary conditions, and as this element has a low chemical intensity and narrow range of special affinities it does not set up any disturbance in living matter Its action in the above experiment is therefore due to the exclusion of the atmospheric oxygen from the cell. The air in the

chamber is one-fifth oxygen at the beginning of the test, while the drop of water in which the material is mounted as well as the cell sap is saturated with the free element.   The result of the exclusion of the oxygen of the air from the cell is not apparent therefore, until this has been used, and may need several minutes.   Carbon dioxide however, has a slightly poisonous action, and it may be seen that movement is inhibited more quickly in this gas than in hydrogen.

59. Influence of Carbon Dioxide upon Protoplasm.   In order to secure absolutely pure carbon dioxide the following method should be used.   Put about 300 g. potassium bicarbonate in a small retort.   Connect the retort with a small gasometer by a tube which has been bent into two acute angles, and the connections made secure.   Fill the gasometer with water that has been boiled to drive off the atmospheric gases with which it is saturated.   The inlet tube of the gasometer should be furnished with a three-way cock.   Heat the retort with a small gas stove or bunsen burner.   Open the three-way cock and allow the gas to pass out
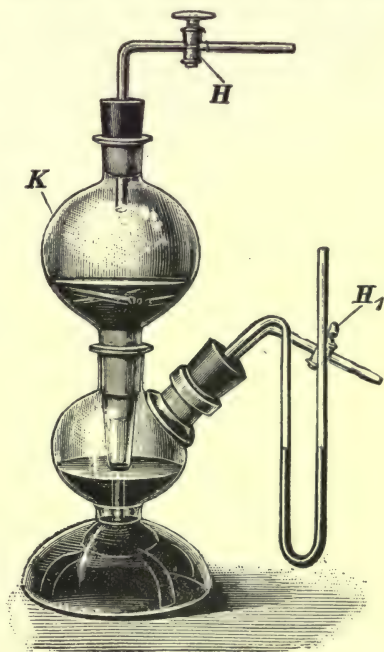
FIG. 23.   Kipp's apparatus for production of carbon dioxide, or hydrogen.   Marble or zinc is placed in the upper chamber, K, and a solution of hydrochloric or sulphuric acid in the lower chambers.   Air is forced in through the tube with the stop-cock H_1 until the acid rises and covers the marble or metal, when the stopcock is closed.   The gas passes directly out through the upper tube with the stopcock H.   Open the stopcock H_1 when sufficient gas has been produced, and the acid will return to the lower chambers.   The manometer connected with the lower chamber is useful in testing the connections and shows the pressure at any time.

until all of the air in the retort has passed off. The decomposition of the potassium bicarbonate takes place in a manner shown by the following reaction: $2KHCO_3 = K_2CO_3 + H_2O + CO_2$. Turn the cock and allow the gas to displace three-fourths of the water in the gasometer. Care must be taken in the heating and the cleaning of the retort that it shall not be broken. Liquid carbon dioxide may be procured in steel cylinders from manufacturers which will replace this material. It should be tested for impurities, and allowed to escape from the cylinder into the gasometer at the proper pressure. After the gasometer has been filled, connect its outlet tube with an Engelmann gas chamber on the stage of a microscope. Two or more microscopes may be placed in series and the same stream of gas allowed to flow through all of the chambers.

Now test the material examined in the last experiment. The streaming movement in *Tradescantia* may be stopped in 1.5 to 2.5 minutes after exposure to the pure gas. Quickly replace the gas with pure air from a small bulb attached to the inlet tube of the Engelmann chambers. Motion is resumed in less than a minute. Great variability in this reaction time will be found, however, in different kinds of material.

Procure a second gasometer or aspirator bottle and make the following mixtures of oxygen and carbon dioxide:

O 25..........CO$_2$ 75
" 20.......... " 80
" 10.......... " 90
" 5.......... " 95

Determine what strength of carbon dioxide is necessary to stop movement. Select a few hairs of *Tradescantia* in which the movement is very vigorous and expose them to a mixture containing 75 per cent. of the carbon dioxide for an hour. Then replace with a mixture containing 80 per cent. Continue until the cells are exposed to the pure gas. In many instances the exposure to mixtures of successive degrees of concentration will allow the

protoplasm to accommodate itself so perfectly that movement may continue for some time in a pure gas.

For the determination of the length of time and concentration in which the pollen will germinate (See chemotropism of pollen) place a number of pollen cells in the proper culture solution on a cover-glass and invert over an Engelmann gas chamber. Expose to the mixtures as above, and note the influence of this compound upon the formation of the pollen tubes. [1]

60. Growth in Oxygen. Make a supply of pure oxygen by heating a mixture of potassium chlorate and manganese dioxide in a retort connected with a gasometer as above, or secure a supply of the gas from a factory in steel cylinders. Test for purity (See text-books of chemistry).

Mount material showing movement of the protoplasm, and then expose to mixtures of air containing increased percentage of oxygen, and to the pure gas. Is the movement accelerated ? Soak a dozen seeds of wheat or corn in water for 24 hours, and then place a half dozen of each in two 10 cm. test-tubes. Push a partition of wire gauze to within 3 cm. of the bottom. Fill the tubes with water and fasten in an inverted position with the mouth of the tube in a dish of mercury. Now displace the water in one tube with air, and the other with pure oxygen. Note results 24, 48, and 72 hours later. Repeat, using different percentages of oxygen. Is the rate of growth of the germinating seedling accelerated by increasing the pressure of the oxygen ?
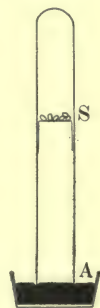
FIG. 24. Respiration tube ; A, mercury; S, germinating seeds resting on wire gauze partition.

61. Influence of Illuminating Gas. The illuminating gas used in cities generally consists of a mixture of marsh gas and volatile petroleum products. This mixture escapes from the pipes and fills up the air-spaces in the soil, displacing the oxygen and exerting its own proper effect upon the plants which send their

[1] Lopriore, G.   Ueber die Einwirkung der Kohlensäure auf das Protoplasma der lebenden Pflanzenzelle.   Jahrb. Wiss. Bot. 28 : 531.   1895.

roots into such soil.   The effect is often noticeable at a distance
of fifteen to thirty meters from the pipes conducting the gas.
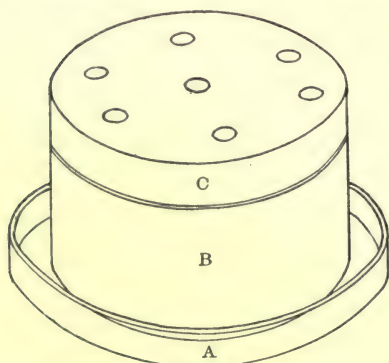
FIG. 25. Zurich germinating dish.   *A,*
shallow dish containing water.   *B,* earthen-
ware vessel for seeds with perforated cove *C.*

Select four vigorous specimens
of tomato or geranium grow-
ing in pots and place them in
an open chamber, ôr out of
doors in a sunny situation.
Fasten a rubber tube to the
tip of a gas jet and carry the
other end to the pot where it
is attached to a metal or glass
tube.   The metal or glass tube
should enter the pot by the
drainage hole in the bottom,
or through an opening at the
side.   Fit a second pot in this

manner, and turn on the gas at the stopcock until a faint odor of
gas can be detected.   Note the appearance of the specimens
from day to day.   Ten days later, disconnect the tubes and com-
pare the root- systems and general aspect of the treated with
other untreated plants.

62. Effect of a Vacuum upon Seeds.   Place a number of healthy
seeds of a half dozen species in a small receiver connected with an
air pump.   Exhaust the air, and turn the stopcock.   More or less
leakage will occur even in the best apparatus, so that it will be
necessary to open the stopcock daily and again exhaust the re-
ceiver to the full power of the pump, which should be shown by
a manometer gauge.   After two weeks' exposure to this partial
pressure, remove to the air, and place in Zurich germinators.
Germinate a number of untreated seeds at the same time for com-
parison.

The above experiment may be performed still better if the seeds
are placed in a glass tube with one end sealed and the other con-
nected with the air pump.   After the air has been exhausted to
the full capacity of the pump, the tube is cut off and sealed at

the same time by the heat of a blowpipe flame without allowing access of air. The specimen can now be kept for an indefinite period before the germination test is made.

63. Influence of Ammonia upon Protoplasm. Mount a leaf of *Philotria*, or a hair showing movement, on a glass slip in the usual manner. Note the rate of movement of the protoplasm by observations on the length of time necessary for a single granule to traverse the length of the cell, or make a circuit of it, or move a given distance as indicated by a micrometer scale. Now run in at one edge of the cover-glass a 10 per-cent. solution of ammonium hydrate, and note the effect upon the movement. After movement ceases remove the ammonia by running in water at one edge of the cover-glass and absorbing it with blotting paper at the other edge. Note the restoration of the movement. Treat another preparation to a concentrated solution of ammonia, and note the effect upon the movement, and the consistency of the protoplasm.

64. Effect of Ammonia Vapor upon Mimosa. Fill a watch glass with ammonium hydrate and place it on a table beside a vigorous, expanded specimen of *Mimosa*. Cover the whole with a large bell-jar, being careful not to give the plant a mechanical shock. Note the character of the movements which follow in a few minutes. Remove the jar and note recovery of plants. Continued exposure will kill the plant as this compound of ammonia is a poison.

65. Nature and Action of Poisons. A large number of substances kill protoplasm when brought into contact with it, and they may bring about the death of a complex organism by disabling some special tissue or group of cells necessary to the continuance of some essential function. According to the manner in which these substances act, they may be classed as oxidizing poisons, substitution poisons, salt-forming poisons, and catalytic poisons.

66. Oxidizing Poisons. The normal process of the release of energy in the prevailing types of the vegetal organism involves a

more or less constant union of oxygen with material held in the
plasma, or of the living substance itself.   As long as this oxida-
tion is attended by renewal with unoxidized fresh material no
disturbance occurs.   If however the supply of food is cut off,
the continued release of energy diminishes the mass of the living
substance and starvation phenomena ensue, comprising other
effects beside that from lack of food, however.   When protoplasm
is brought into contact with compounds readily yielding their
oxygen it unites with this substance, burning up very quickly.
It is to be noted however that this oxidation is not simply an in-
crease of the processes normally in action in the plant, but new
oxidations are set up which reduce the whole machinery of the
living matter to a form from which nearly all energy has been
lost.   The consistency and appearance of the injured protoplasm
will be unlike in the two instances.

67. Starvation.   Place some fresh filaments of *Spirogyra* in a
deep dish filled with distilled water entirely free from sediment.
Allow it to remain in this liquid for several days, and note general
appearance from day to day.   Two weeks later, or as soon as the
filaments have begun to deteriorate, examine the structure of the
cells with a magnification of 300 to 600 diameters.   Reserve ma-
terial will be seen to have disappeared, the nucleus will have lost
its sharp contour and drops of oil will be apparent.

68. Oxidizing Effect of Potassium Permanganate.   Place a
number of healthy filaments of any species of *Spirogyra* in a .2
per-cent. solution of potassium permanganate and keep under
observation with the microscope for ten minutes.   Wash in clear
water and examine ; if alive restore to a culture dish and note
appearance a day later.   Treat the same preparation with a .5
per-cent. solution, and note results ten minutes after immersion.

69. Oxidizing Effect of Potassium Chlorate.   This compound
causes oxidation in living matter only, under ordinary circum-
stances, while the permanganate attacks all organic matter.   Treat
Spirogyra with .5 per-cent, 1 per-cent. and 3 per-cent. solutions
and note results in ten and twenty minutes after exposure.

70. Oxidizing Effect of Hydrogen Peroxide. Repeat 69 using .5 per-cent., 1 per-cent., 3 per-cent., and 6 per-cent. solutions of hydrogen peroxide.

71. Catalytic Poisons. A number of compounds of the hydrocarbons which are not acid or basic, or very active chemically, are poisonous by their presence ; inducing chemical changes in the plasma without participating in this action themselves. The intensity of the action of compounds of this class seem to increase with their molecular complexity. The influence of these substances seems to consist in setting up new molecular disturbances in the compounds with which they come in contact, without actually entering into any chemical combination with them. Their influence is likened to the behavior of a row of blocks set up in such manner that the falling of the first one throws down the second, which in turn knocks down the third until the whole row is prostrate. The impulse communicated to the first molecule is communicated to the others until the entire mass is affected. Among the catalytic poisons are ether, alcohol, chloroform, chloral, carbon disulphide and many others.

72. Effect of Ether and Chloroform on Movement. Pour enough ether into a wash bottle to form a layer 5 cm. deep on top of an equal amount of water previously added. Connect the outlet with one of the tubes of an Engelmann gas chamber, and run a rubber tube from the other side of the gas chamber to a filter pump or an air pump. The tubes in the wash bottle should be arranged so that air will be drawn in through the wash bottle by a tube extending below the surface of the ether. The stream of air bubbling up through this becomes charged with ether vapor and is then drawn through the chamber. Mount a specimen showing movement of protoplasm, on a cover-glass, and invert over opening of gas chamber. Open a pump and allow a stream of vapor to pass through. Note cessation of movement. Open the chamber and allow access of air. Note resumption of movement. Repeat with chloroform.

5

73. Effect of Chloroform upon Mimosa. Place a sponge saturated with chloroform near a vigorous expanded specimen of *Mimosa* and cover with a bell-jar, being careful not to give the plant a mechanical shock. Does the action of the vapor cause movement? After ten minutes remove the jar and apply shock stimuli. Determine the length of the period necessary for the plant to recover irritability to mechanical stimuli. Allow a second specimen to remain under the bell-jar for a day with chloroform vapor and note results.

74. Effect of Chloroform upon Oxalis Leaves. Add 1 cc. of chloroform to 200 cc. of water in a bottle and shake. Cut a leaf of *Oxalis* into narrow strips and place them in the liquid. The length of time necessary for the chloroform to kill the tissues may be determined accurately since the leaf assumes a dingy yellow color upon death.

75. Degree of Molecular Complexity and Intensity of Poisonous Action. The series of alcohols of the lower paraffins affords a convenient means of demonstration of the correspondence of molecular complexity and intensity of poisonous action. The formulæ for some of the alcohols are as follows :

Methyl, $H—CH_2OH$.
Ethyl, $CH_3—CH_2OH$.
Propyl ; norm., $CH_3.CH_2—CH_2OH$.
Propyl ; iso., $CH_3—CH.OH—CH_3$.
Butyl ; norm., $CH_3.CH_2.CH_2.—CH_2OH$.
Butyl ; iso., $\dfrac{CH_3}{CH_3}{>}CH—CH_2OH$.
Butyl ; tertiary, $\dfrac{CH_3}{CH_2}{>}COH—CH_3$.
Amyl ; norm., $CH_3.CH_2.CH_2.CH_2.—CH_2OH$.

Prepare two series of six small dishes in which colonies of *Spirogyra* may be grown. Make solutions of ethyl alcohol and of methyl alcohol containing the following concentrations ; normal, $\frac{2}{3}$ normal, $\frac{1}{2}$ normal, $\frac{1}{4}$ normal, $\frac{1}{8}$ normal and $\frac{1}{32}$ normal. Nor-

mal solutions are prepared by adding the hydrogen equivalent of the reagent in grams to a liter of distilled water (78). The " normal " solution of common salt used by animal physiologists however contains .6 g. salt in a liter of water (Sterling). Place small colonies of *Spirogyra* in every dish, and add enough of the above solutions to fill the dishes to a suitable level. Cover loosely and set in sunlight. Examine material a day later. Determine, by plasmolysis tests and general appearance, the solutions causing death and tabulate results. Compare the action of the two alcohols and note their relative complexity of chemical structure. A normal solution of ethyl alcohol is made by adding 46 grams of absolute alcohol to a liter of distilled water. Normal solution of methyl alcohol is made by adding 32 grams to a liter of distilled water.[1]

A remarkable instance of the capacity of a structure resisting penetration by alcohol (ethylic ?) is that cited by Barnes who kept sporocarps of *Marsilia quadrifolia* immersed in a 95 percent. solution for six years, yet when these where washed and immersed in water the spores germinated normally. The spore-coverings were doubtless impervious to the alcohol, and the spores must have lived the entire period with only a minute supply of oxygen.[2]

76. Nature of the Action of Anæsthetics. A number of the catalytic poisons when applied to the organism in dilute solutions, or in low concentration, render the organism insensible to the various forces which usually act as stimuli, as illustrated by the action of chloroform on *Mimosa*. The manner in which anæsthetic reagents affect living matter is not clearly made out. Deductions from the results of narcosis of the higher animals may not be given very wide significance, since in such organisms specialized tissues are affected and adaptive or regulatory processes are set up, the products of which may be quite unlike those of simple organisms. Anæsthetics undoubtedly depress the

[1] Tsukamoto. On the poisonous action of alcohols upon different organisms. Journal Coll. of Science : Japan, 7 : 269. 1895.

[2] Barnes, C. R.   Vitality of *Marsilia quadrifolia*. Bot. Gazette, 20 : 229. 1895. Also Plant World, 2 : 140. 1899.

contractile, or motile functions of the protoplast, but not all of the other functions, especially those essentially metabolic in their nature. Thus it has been found by recent investigations that plants show an increase in respiratory activity under the influence of anæsthetics.[1] The probability may be admitted that not all substances which have a narcotic or anæsthetic action upon living matter, are catalytic in their action ; direct chemical combination may be made with some of the substances in the cell.

77. Poisons which Form Salts. Some substances are poisonous to protoplasm by forming salts with its constituents. Among these are acids, mineral bases, and salts of heavy metals. The organic acids are not so powerful in their action in general as the inorganic, but both are very deadly in the minutest quantity to certain forms of plants, especially algae.

The poisonous effect of the mineral bases may sometimes be due to the purely physical action, such as osmotic attraction by which water or organization may be withdrawn from the colloidal mass of living substances so extensively as to cause its disorganization.

By the recent researches of True and Kahlenberg it has been found that the toxic action of dissolved salts and acids depends to a great extent upon their dissociation when put into solution in water. The undissociated molecules will exert their own proper effect upon the protoplasm, with the added effect of the separate ions of the dissociated portion of the substance. Furthermore, the ions may combine to form complex ions with a still different and separate effect. Mercuric cyanide is an example of a substance which is not dissociated in solution. Its poisonous effect is therefore due solely to the proper chemical effect of its entire molecules. Lupines were found to survive with their roots immersed in a solution of $\frac{1}{102400}$ gram molecule per liter. Silver nitrate, on the other hand, is strongly dissociated in solution, acting through its ions. The roots of the lupine will endure only $\frac{1}{409600}$ gram molecule per liter of this substance.

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[1] Morkowine, N.   Recherches sur l'influence des anesthésiques sur la respiration des plantes.   Rev. Gen. Bot. 11 : 289, 341.   1899.

A comparison of the effects of a substance when dissociated into simple ions, and into complex ions is afforded by copper in the form of a sulphate, and in a modified Fehling's solution. The root of lupine will not survive in a solution of copper sulphate more concentrated than $\frac{1}{51200}$ gram molecule per liter while it will live in a modified Fehling's solution (copper sulphate, sodium hydrate and sugar) of a concentration of $\frac{1}{400}$ gram molecule per liter.[1]

78. Toxic Action of Substances in an Ionic Condition. The toxic action of a substance in an ionic condition may be determined by testing the effect of two dissociable salts in which it occurs. Thus if a dilute solution of sodium chloride is harmless while one of hydrochloric acid is fatal, the poisonous action is plainly due to the hydrogen present, since it is known that chlorine ions are harmless in such solutions. Then again if sodium nitrate is harmless in dilute solutions and nitric acid is fatal, the action of the latter is to be ascribed to ionic hydrogen.

Solutions of hydrochloric, nitric, and sulphuric acids are practically completely dissociated when an amount of these compounds in grams equal to their molecular weights divided by the number of H atoms (one gram equivalent) is added to one liter of distilled water ; and since the Cl, NO_3 and SO_4 ions are relatively harmless when combined with sodium salts, it may be concluded that the toxic effect of such solutions is due to ions of hydrogen, and that this effect will be generally the same in the three acids (See 75, normal solutions).

79. Toxic Effect of Hydrochloric Acid. Germinate some seeds of *Lupinus albus* by soaking in distilled water for a day, then place in cotton wool until the roots are about 2 cm. in length. Prepare a few small beakers of glass by cleaning them thoroughly and washing with distilled water. Fit to each beaker a cork plate which sets over the top like a lid. Push through the cork a clean glass rod which reaches half way to the bottom of the beaker. Fit on this rod a second cork of half the size of the upper one.

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[1] Kahlenberg and True. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gazette, 22 : 81. 1896.

When ready to start the test the seedlings are fastened to the circumference of this cork by means of pins thrust through small split corks holding the seedlings, and the glass rod is pushed up or down to allow the roots to be immersed over a greater part of their length.   Have the beaker half full of a solution of a strength of $\frac{1}{3200}$ gram equivalent per liter.   Make up a second solution of half of the above concentration, also a third and fourth of the same strength.   Fasten several seedlings in each beaker.   Make a fine India ink mark by means of a thread saturated with the fluid and held taut by a pair of calipers, at a distance exactly 15 mm. from the tip of each root.   Measure the amount of elongation or growth of this apical portion of the root daily.   The data thus obtained will determine the effect of the acid upon protoplasm, and will also fix the concentration producing fatal results.[1]

80. Toxic Effect of Silver Nitrate.   Make up fractional normal solutions of silver nitrate and determine the degree of concentration in which the roots of *Zea*, or *Phaseolus* may survive (78).

81. Effect of Oxalic Acid.   Repeat 79 with oxalic acid as an example of the organic acids.

82. Toxic Effect of Potassium Hydrate.   Repeat 79 with solutions of potassium hydrate made up as above.[2]

83. Substitution Poisons.   Certain substances may be classed as substitution poisons, and comprise some of the sulphur compounds and many nitrogenous compounds.   The substitution poisons attack chiefly the amido and aldehyde groups in living matter.   Many of these substances bear special relations to the higher animals, by affecting specially differentiated masses of tissue.   An example of this is afforded by hydrocyanic acid, which is much more highly poisonous to the higher animals than to the lower forms.

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[1] Heald, F. D.   On the toxic effect of dilute solutions of acids and salts upon plants.   Bot. Gazette, **22 : 125.**   1896.

[2] Kahlenberg and True.   On the toxic action of dissolved salts and their electrolytic dissociation.   Bot. Gazette, **22 : 81.**   1896.

84. Toxic Effect of Phenol. Make up a .5 per-cent. solution of carbolic acid (phenol) in distilled water, and drop into it fresh strips of leaves of *Oxalis*, noting length of time necessary to produce death as indicated by discoloration of the leaf. Place some filaments of *Spirogyra* in a solution of equal strength and note results in three hours and a day later. Note changes in cell structures. Make a series of decreasing intensity and determine in what concentration the alga may survive. The low conductivity of phenol shows that practically no dissociation occurs. Phenol is a poison to the higher animals by inducing paralysis of the nerve centers, and also works direct injury to the cells with which it comes in contact.[1]

85. Toxic Action of Phloroglucin. Repeat 84 with solutions of phloroglucin and fix the limit of toxic acid of this substance. Compare its action with that of phenol.

86. Toxic Action of Formaldehyde. Place a small lot of *Spirogyra* in a culture dish containing water from a stream, and sufficient formaldehyde to make a .01 per-cent. solution. Examine from day to day and note length of time the alga may endure this concentration. Make similar tests with solutions more and less dilute. Test also with seedlings of lupine as in 75.

87. Poisonous Proteinaceous Substances. A large number of proteinaceous compounds secreted by plants are well known as deadly poisons to the higher animals. Many of them are unstable and act as substitution poisons upon protoplasm. Their action upon the higher organisms among animals consists of special disturbances of the nerve centers in many instances. Such compounds do not pass the cellulose and protoplastic membranes of the plant with great readiness, but in experimental tests in plant cultures disintegration often ensues with the result that simpler, more easily dialyzable, substances are formed that exert a direct, or indirect toxic action.

[1] True and Kunkel. The poisonous effect exerted on living plants by phenols. Bot. Centralbl. **76** : 289, 321, 361. 1898.

A few of the alkaloids are capable of exerting a toxic effect upon plants by direct action. In general however, the alkaloids are exceedingly divergent in their action ; these substances are nitrogenous, basic and very complex. It is supposed that their deleterious influence is due to the union of the bases with the active proteins of the cell, thus setting up most serious disturbances.[1]

88. Toxic Action of Alkaloids. Place hairs of *Tradescantia* or filaments of *Spirogyra* in .1 per-cent. and 3 per-cent. solutions of caffeine on glass slips, and note changes in the cell as seen with a magnification of 4–500 diameters. Make similar tests with cocaine.

Dissolve 1 part of sulphate of quinia in one thousand parts of distilled water, and test the influence of this solution upon motile zoöspores and hairs with streaming movements of the protoplasm.

89. Self-poisoning. The alkaloids and other poisonous substances produced by the metabolic action of the plant may serve the incidental purpose of protecting the plant from the ravages of grazing animals, but they are usually by-products which the organism translocates to some portion of the body which is cast off, or they are united with other substances to form insoluble or innocuous compounds. Disturbance of this action by the plant may result in pathological conditions. Furthermore, substances not ordinarily known as poisons may act as such by their destructive action upon essential constituents of specialized cells. An example of this is to be found in the increased production of oxidase in the leaves of the tobacco plant, in which the increased enzyme is not kept to its usual function, but attacks the chloroplasts and disintegrates the chlorophyl, inducing a pathological condition of the leaf. Doubtless many other pathological phenomena are also due to a lack of proper automatic control of metabolic products owing to unusual cultural conditions (See oxidases).

90. Acclimatization to Chemical Action. A summary of the results obtained in the previous experiments shows that the protoplasm of different organisms has a different capacity for resistance

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[1] Loew, O.   Ein natürliches System der Gift-Wirkungen.   1893.

to the action of chemical agents. The capacity for endurance may be increased by successive exposures to a series of solutions, beginning with one of low concentration, and passing in successive periods to higher, or more concentrated ones. Thus Lopriori found that while the streaming movements of protoplasm were inhibited by exposure to an atmosphere of one part oxygen and four parts carbon dioxide, yet if the plant were first exposed to a mixture of 25 parts of oxygen and 75 of carbon dioxide for a time, it might then be brought successively into atmospheres containing 80, 85, 90, 95 and even 100 parts of the gas without immediate injury. The acclimatization of a plant to any trophic agent of course carries with it a readjustment of the three critical points, the optimum, maximum and minimum. The acclimatization of the organism to a new intensity of one agent generally affects the critical points in relation to others.

91. The Changes which Ensue in Protoplasm During Acclimatization. It is possible that protoplasm ceases to manufacture any one of the substances illy affected by the chemical agent, replacing it by others not so readily disintegrated or formed into new compounds. The endurance of *Marsilia* has already been pointed out as an instance of the efficiency of a protective covering as a shield against chemical reagents. It is possible that this method may be employed in some cases of acclimatization.

92. Chemotaxis. Many organisms have acquired the power of adaptive movement in response to the presence of chemical substances serving as food, or as accessory reproductive devices. Such movements are exhibited by roots in their movements through the soil, by the absorbing organs of the lower forms, by the pollen tubes of the higher plants, and by nearly all free motile organisms. The connection between the molecular structure of the stimulating substances and the amplitude of a response, has received but little investigation ; it seems quite probably however, that the chemotactic influence of many compounds may be attributed to the action of the dissociated ions, although in this as well as in toxic action the undissociated molecules of the same

substances also exercise an effect. Moreover many trophic sub-
stances such as sugars, asparagin, etc., which do not undergo
electrolytic dissociation produce chemotactic effects. Every
organism has acquired the power of reacting to certain substances
which are of importance in its existence. The mechanisms of re-
sponse may be set in action by other substances of related chemical
structure, or other forces, of which the plant had had no previous
experience.[1]

93. Relation of the Organism to Trophic and Other Compounds.
The three critical points may be noted in the relation of a plant
to trophic substances. It is to be said however that the minimum
of intensity is generally very low. A correlation is to be found
between the optimum and maximum and the irritable influence
of a substance. When a free-moving organism finds itself in a
medium at a point where any trophic substance is below the
optimum in concentration, or below a certain standard of experi-
ence, it begins to move toward the point where the concentration
is greater. This positive action is sustained until the point is
reached where the tonic optimum is reached. If on the other
hand, the organism should be under the influence of a concentra-
tion above its optimum, or standard of experience, it will move
away from this concentration toward a point where this optimum
may be attained. It appears probable that the positive chemo-
tactic movement is due to the attractive power of the ions of the
radicle, and that the negative action is due to the hydrogen ions
in dissociated substances. The positive response is continued
until the repelling power of the hydrogen overbalances the con-
trary influence of the radicle, and then the negative reaction is
shown. It has been held by some investigators that the repel-
lant power of concentrated solutions was due to osmotic action.
The influence of the non-trophic substances is most varied. Thus

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[1] In this connection see, Jennings, H. S. On the movements and motor reflexes
of the flagellata and ciliata. Amer. Jour. Physiol. 3 : 229. 1900. And, Garrey. The
effects of ions upon the aggregation of flagellated infusoria. Amer. Jour. Physiol.
3 : 291. 1900.

to some of these the organism exhibits only negative action and moves out of the sphere of their influence as a reaction. Again the organism may not be repulsed by the highest concentration of the substance even when poisonous.

The threshold of stimulation lies at a very low degree of concentration. After the organism is under the influence of any chemical agent however, the additional intensity necessary to constitute a stimulus will increase with the concentration already existing, in accordance with Weber's law. Thus if an organism is in distilled water it will react to a much smaller intensity of action of sugar than if it were in a one-per-cent. solution of this substance.

94. Chemotaxis of Antherozoids of Ferns. Secure numbers of prothallia of *Adiantum,* or some species which produces great numbers of antheridia in comparison with the archegonia. A crop of prothallia may be provided by sowing spores in a moist chamber in a greenhouse two months before needed. Examine from time to time and when the antheridia seem about mature take a few of the prothallia from the soil and wash clean. Place on a glass slip and cover with a small circle of glass, which should be supported at the edges by small bits of glass or hairs. Draw rain water through the preparation several times in order to wash free from organic acids. Malic acid is present in the cells, and if any have been ruptured the free acid would interfere with the success of the experiment. Draw out some fine capillary tubes until they are not more than .2 mm. in diameter. Cut in sections about 8 mm. in length and close one end by fusion. Make a one-tenth per-cent. solution of malic acid or sodium malate in a small dish and lay the tubes in it. Set under the receiver of an air pump and exhaust. The expansion of the air contained in the capillary tubes will allow some of it to escape and when the dish is removed from the receiver, some of the solution will run in to take its place. Select one of the capillary tubes and rinse it lightly in water for a moment, then thrust the open end under the edge of the cover-glass into the water. The

antherozoids will have escaped and be moving rapidly around in the liquid. Remove the capillary tube one minute later, and examine under the microscope for antherozoids.[1] Make a second test and allow the capillary tube to remain in the water with the motile bodies for five minutes. Compare the number of the antherozoids which have entered the tube with that of the first test. The archegonial cells contain salts of malic acid, and these substances serves to attract the swimming antherozoids, and thus secure fertilization of the egg-cells.[2] Hairs of *Heracleum* containing malic acid may be used instead of the glass tubes.

95. Chemotactic Movements of Bacteria. The stimulating power of food substances may be seen if the movements of bacteria are studied. Boil a pea or two for a few minutes, then put it in a few cc. of water in an open dish and allow it to stand for a day or two. Spores of *Bacterium termo* floating in the air will have fallen into the liquid and developed great numbers of colonies and individuals. Filter some of the liquor containing the bacteria through glass wool to obtain a solution in which individual bacteria are swarming. Now place a large drop of the culture on a glass slip. Prepare capillary tubes as in the previous experiment, but fill them with a one-per-cent. solution of extract of beef (commercial preparation). Thrust the ends of one or two of these tubes in the bacterium culture drop for a few minutes. Examine with a microscope and note the number of the organisms that have been attracted to the beef solution and entered the tube.

96. Chemotropic Movements of Pollen Tubes. The demonstrations with bacteria and antherozoids showed that these bodies actually move toward a spot in which the stimulating compound is found in an optimum intensity. In certain instances the plant structure does not move, but directs its growing parts toward the optimum intensity of the stimulating agent. Add 1 g. of gelatine

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[1] Pfeffer. Locomotorische Richtungsbewegungen durch chemische Reize. Untersuch. a. d. Bot. z. Tübingen. 1 : 363. 1884.

[2] Buller, A. H. Contributions to our knowledge of the physiology of the spermatozoa of ferns. Annals of Botany, 14 : 543. 1900.

and 4 or 5 g. cane sugar to 50 cc. distilled water.   Warm until a homogeneous solution is obtained.   Place a drop of this solution on a glass slip and when cold add a number of pollen cells of *Narcissus, Fritillaria,* or *Lathyrus.*   Cover with a thin circle of glass and set in a moist chamber kept as near 18° C. as possible. Examine eight or ten hours later, and also the next day.   Note the direction taken by the pollen tubes.   All seem to be pointed
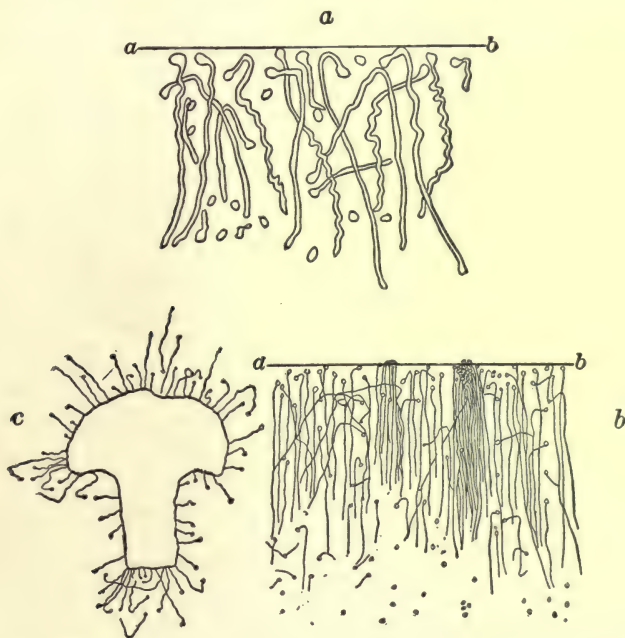
FIG. 26.   Chemotropism of pollen tubes.   The lines *a* to *b* represent the edge of a cover-glass under which are pollen tubes.   In *a,* pollen of *Narcissus tazetta* in a seven per-cent. solution of sugar is seen to be negatively chemotropic to the air at the edge of the cover-glass.   In *b* negative chemotropism of pollen tubes of *Cephalan-thera pallens* in seven per-cent. sugar solution is shown, about 20 hours after beginning of experiment.   *c,* stigma of *Narcissus tazetta* with pollen tubes impinging on its surface.   After Molisch.

toward the center of the preparation, or away from contact with the air.   Make a second preparation and treat it as the first but seal the edges of the cover with vaseline.   Compare the behavior

of the tubes in the two instances.   Pollen tubes are apochemo-
tropic to the amount of oxygen in the air (20 per cent.) and direct
their tips away from atmospheres with this pressure of the gas.
Sealing the preparation with vaseline should exclude the gas and
allow the tubes to grow in all directions.

97. Chemotropic Stimulation of Stigmas for Pollen Tubes.
Make a culture medium for pollen grains as above, using only
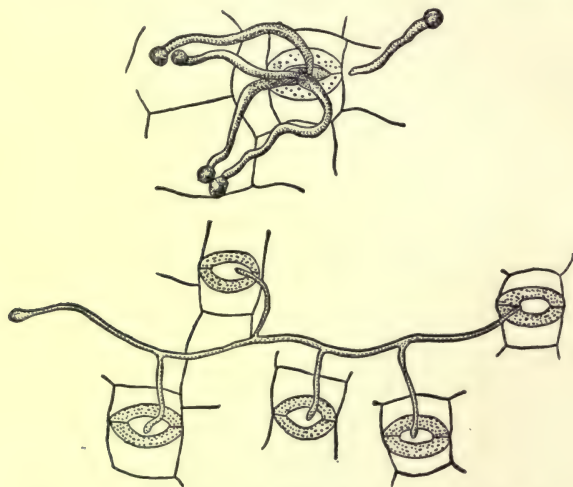
FIG. 27.   Chemotropic reactions of pollen tubes in penetrating the stomata of
leaves injected with sugar.   Upper figure, hyphae of *Phycomyces nitens* penetrating
leaf injected with two per-cent. dextrin solution, 25 hours after beginning of experi-
ment.   × 20.   Lower figure, branching hyphae of *Penicillium glaucum* penetrating
stomata of leaf of *Tradescantia* 25 hours after beginning of experiment.   × 70.
After Miyoshi.

so much sugar as may be sufficient for the particular species
under test (3 to 15 per cent.).   Cut a small section from the surface
of the stigma of the same plant and place near one edge of the
culture fluid.   Add a number of pollen grains, ring one with
vaseline and leave a second open.   Note the directions of the
pollen tubes a day later.[1]

[1] Lidtforss.   Ueber den Chemotropismus der Pollenschläuche.   Ber. Deut. Bot.
Gesell. **17** : 235.   1899.

98. Chemotropic Reactions of Mucor or other Moulds to Sugars.
Prepare a two per-cent. solution of cane sugar and place a leaf of
Tradescantia in it.   Set on the plate of an air pump, and cover
with a small receiver.   Exhaust the receiver.   This will draw the
air from the leaf and inject the intercellular spaces with sugar.
Remove the leaf, wash quickly in water and wipe dry.   Place the
leaf with the under side uppermost on a plate in a moist chamber.
Now sow spores of mould on the leaf.   Examine daily with the
low power of the microscope and note direction taken by the
hyphae formed by the germination of the spores.[1]

**99. Influence of Chemical Stimulation upon Developmental Proc-
esses.**   Chemical substances in the medium in which an organism
lives may affect the growth and development of various organs in
many ways.   The stature and form of the organs, the structure
of the tissues, the rate and amount of growth, and the perform-
ance of reproduction may be profoundly modified by the presence
or absence of chemical agents, in a relation wholly due to a stimu-
lating effect, and entirely beyond their nutritive or trophic value.

Richards has found that such substances as sodium fluoride,
zinc sulphate, sodium silicate, cobalt sulphate, cocaine, and mor-
phine in minute quantities cause an acceleration of growth both
as to rate and ultimate performance in various fungi.   Schulze
found that such substances as corrosive sublimate, iodine, bro-
mine, and arsenious acid increase the activities of yeast in fer-
mentation.   Ono traced the influence of various salts upon algae
and fungi, and many investigations upon the subject are cur-
rent.[2]

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[1] Molisch.   Zur Physiologie des Pollens mit besonderer Rücksicht auf die chemo-
tropischen Bewegungen der Pollenschläuche.   Sitzungsb. d. Akad. d. Wiss. Wien.
102 ; 423.

Miyoshi, M.   Ueber chemotropismus der Pilze.   Bot. Zeitung. 52. 1.   1894.

[2] Richards, H. M.   Die Beeinflussung des Wachstums einiger Pilze durch chem-
ischer Reize.   Jahrb. Wiss. Bot. **30** : 665.   1897.   And, The effect of chemical irri-
tation on the economic coefficient of sugar.   Bull. Torr. Bot. Club, **26** : 463.   1899.

Schulze, H.   Ueber Hefegifte.   Arch. f. Ges. Physiologie. **43** : 517.   1888.

Ono, N.   Jour. Coll. Science Imp. University, Tokyo.   **13** : 143.   1900.

Clark, J. F.   Dissociation and toxic effect.   Journ. of Physical Chem. **3** : 263.
1899.

The form and size of the leaves and stems of plants growing in soil rich in alkali or saline matter are deeply affected by these substances. Sexual and asexual reproductive processes may be called up or suppressed by the influence of different compounds. Recent investigations by Loeb show that after the stimulation of eggs of some of the lower animals by means of certain magnesium and sodium compounds the eggs would develop as if they had been fertilized or received the male reproductive element, and Wilson has produced important variations in the primary proc- esses of division of nuclei, and cleavage of the cytoplasm, by the use of various chemicals.[1] Among the most singular changes in form as a result of chemical action are galls or excrescences formed on various plants as the result of a puncture and deposit of eggs by insects and other animals. The deposition of the egg, and the development of the larvae is undoubtedly accompanied by the formation of an enzyme by the parent and its deposition with the egg, or by its formation by the egg or larvae. In any case the action of this enzyme exercises a stimulating effect that causes the host plant to construct various abnormalities known as galls.

The rosettes formed on the tips of branches of willows, and the galls of the oak are familiar examples of such action. Kraemer has found that the changes in the tissues of the gall do not cease with its separation from the plant on which it is borne, but that the larvae of *Cynips* inhabiting the gall of the oak may induce changes in the character of the cell contents, by which gallic acid is manufactured at the expense of the starch.[2]

[1] Livingstone, B. E.   On the nature of the stimulus which causes the changes of form in polymorphic green algae.   Bot. Gazette. 30 : 289.   1900.

Duggar, B. M.   Physiological studies with reference to the germination of certain fungous spores.   Bot. Gazette. 21 : 38.   1901.

Loeb, J.   Artificial parthogenesis in sea urchins.   Science, 11 : 612.   1900.

[2] Kraemer, H.   Origin of tannin in galls.   Science, 12 : 583.   1900.

Küster, E.   Beiträge zur Gallenanatomie.   Flora, 87 : 117.   1900.

IV. RELATIONS OF PLANTS TO WATER

100. Water as a Factor in Living Matter. Water is the most abundant constituent of living matter, serving as an organizing fluid for the colloidal matter, and as a solvent for the crystalloids, making a medium of exchange between the different organs of the protoplast, and serving most important uses in all metabolic processes. It is also to be considered as a nutritive substance, yielding its constituent elements for the construction of essential compounds of living matter. It serves as a medium for the introduction of food-material into the protoplasts, and their conduction from one part of the body to another. In addition the water imbibed by cell-walls determines their ductility, elasticity, and flexibility, while the rigidity of the entire body of the plant is more or less dependent upon the amount of water absorbed and held inside of the membranes of the protoplasts. The absorption, transpiration, guttation and conduction of water by the plant will receive special attention in the sections devoted to these functions. The proportion of water in protoplasts may be as much as 98 per cent. of their gross weight, and living matter may exist under certain conditions in seeds, spores, etc., with only five or six per cent. of this liquid. Reduction below the last-named figure may result in disorganization. The fatal proportion below which death ensues varies greatly in different structures however. It is probable that leaves may not live with less than 35 per cent. of their weight of water. The optimum point is far above this, while the maximum can hardly be distinguished, since it is rarely possible to induce a protoplast to acquire too much water. The minimum, or point at which the ordinary activities of the cell cease and rigor sets in is not so well defined in the relations of protoplasm to this agency as to others. At a certain point, which might be termed the minimum, most of the activities cease and a partial rigor sets in, but respiration and

some forms of metabolism continue until desiccation reaches a point where disorganization ensues.

These manifold relations of water to the organism give it a potent influence in determining the form and structure of the organs. The size of the individual, stature of the leaves, and structure of the organs of absorption and transpiration respond directly to the environmental water relations of the plant.

101. Effect of Desiccation upon Movement of Protoplasm. Select a leaf of *Philotria* in which rapid movement of the strands of protoplasm may be seen. Remove the cover-glass, exposing this aquatic leaf to the air for a half hour. Re-cover, and add a drop of water; note time necessary for resumption of movement. Test the extent to which this desiccation may be carried and the movement resumed.

102. Resistance of Seeds to Desiccation. A striking test of the power of seeds to undergo desiccation may be made if a number of seeds of the pea, wheat, corn and radish are placed in a small glass tube sealed at one end. They should have been previously dried in the sun for a week. Now connect the tube with a mercurial air pump and exhaust to the full capacity of the pump. With the pump still in action, seal up the tube by cutting across, or fusing it with a bunsen flame, taking care not to heat the seeds unduly. A month later break the tube, and place the seeds in a second tube and seal as before. Take the seeds from the second tube at the end of a month and test germinating power. Gather a half dozen kinds of seeds from tender herbaceous plants, growing in moist shaded situations, and place them in a desiccating chamber using sulphuric acid to absorb moisture. Test the germinating power of the desiccated seeds two weeks later. Seed pans or some form of germinator should be used for these tests (See also, Effect of vacuum upon seeds).

103. Hydrotropic Reactions. Some plants have acquired a specific irritability to moisture which enables them to direct absorbing, or other organs toward a greater intensity of this substance if the plant is not receiving its maximum supply, or away

from it in certain instances in which dryer atmosphere or soil is desirable. Roots, pollen tubes and rhizoids are found to be prohydrotropic, while the sporangia of some of the fungi and myxomycetes are apohydrotropic. The latter reaction, which is shown by *Mucor stolonifer*, *Phycomyces nitens* and *Coprinus velaris*, is evidently an adaptation for carrying spores as far as possible from the moist surroundings of the plant, and permitting their dissemination by the wind. Roots placed in currents of water generally react to the force of the flowing water by bending toward or away from the source of the current. Similar reactions are shown by plasmodial masses of the myxomycetes. The curvature in response to a current of water is probably due to the mechanical force received, rather than to any quality of the water and is termed *rheotropism*.

104. Prohydrotropism of Roots. Cover the outside of a small glass funnel, or one of porous earthenware with wet filter paper. Stop the opening in the funnel with a plug of cotton wool and fill with moist sand, rounding it up on top. Imbed kernels of corn around the edge of the funnel in the sand, directing the apices of the seeds outward and over the edge of the funnel. Cover the kernels with a circular piece of filter paper. Place the funnel in the mouth of a bottle filled with water. Set the preparation under a bell-jar. As the corn grows the roots will escape over the edge of the funnel into the air. If the air in the bell-jar is saturated with moisture the roots will grow directly downward. If the air has been kept only moist enough to prevent desiccation however, the roots will bend toward the filter paper, the lower edge of which is immersed in the water in the bottle. The proper degree of ventilation may be secured by raising one edge of the bell-jar. This reaction is a delicate one and a second or third trial may be necessary to adjust all the conditions properly (Arthur, Exp. in Veg. Physiol.). The sand may be replaced by a shallow germinating dish in which the seeds are covered with filter paper, and openings are provided for the exit of the roots (Fig. 28).

105. Reactions of Plasmodia to Water; Hydrotropism. A naked mass of protoplasm offers some experimental features of advantage over those of a vegetable structure encased in cellulose membranes. The plasmodial forms of myxomycetes may be found in the spring, summer and autumn on decaying logs, leaves, and stumps in forests, or they may be grown from spores of *Trichia*, *Arcyria*, *Stemonitis*, *Didymium* or other convenient forms. Plasmodia collected in a forest may be preserved and taken into the laboratory by lifting a fragment of the material on which they are growing, and placing in a jar or closed tin box. Spores may be germinated on pieces of the material on which they were grown in a moist chamber at temperatures of 20 to 25° C.

FIG. 28. Apparatus for the demonstration of hydrotropism of roots. *h*, *h*, wide-mouthed bottle. *s*, sand. *kk*, seedlings. *p*, showing arrangement of filter paper. *tr*, *tr*, openings in dish. *w*, *w*, curving roots. After Molisch.

Cut a piece of filter paper the size of a microscopic slide and saturate it in water, then lay it on a slide. Now coat a section at one end inclusive of about three sq. cmm. with liquefied gelatine. Transfer a plasmodium to the filter paper about the center of the slide. Place the preparation in a small moist chamber, ventilated in such manner that the filter paper will dry slowly through a period of several hours or a day. The moisture will be retained longer by

the gelatine and the plasmodium will be found to move to-
ward it.[1]

106. Rheotropic Reactions of Plasmodia.   Secure specimens of
plasmodia growing on leaves or decaying wood as in 103 and
bring into the laboratory with as little disturbance as possible.
Lay the material on the bottom of a flat dish.   Arrange a beaker
of water near by, and place a strip of filter paper with one end
in the water and the other brought over the side of the beaker
and resting on the material near the plasmodium.   The plas-
modium will be found to move toward and upward along the
filter paper.   It is attracted or stimulated by the current of
water, and responds by moving against it.   The mechanism by
which this is accomplished is not easily explainable.   The above
test should be made in a dark room to avoid phototropic reactions.

**107. Influence of Water and Water Va-
por on Form.**   The long continued expo-
sure of a species to a habitat rich in mois-
ture, or to arid conditions will result in
adaptations suitable to the endurance of
these water relations.   The change of a
plant from a land to an aquatic habitat re-
sults in finely divided or ribbon-shaped
leaves, while the species living in arid re-
gions may develop thick succulent foliage
or other forms suited to the storage or
conservation of the limited water supply.
These outward changes in form are ac-

FIG. 29.   Leaves of *Ra-
nunculus delphinifolius: W,*
growing submerged in the
water: *L,* growing from the
portion of the stem above the
surface.   After Goebel.

companied by many internal adaptations.   Many species are so
elastic that they are capable of producing organs adapted to ex-
treme conditions on the same individual.

**108. Form and Structure of Organs in Water and Watery
Vapor.**   Grow seedlings of corn for a week in a germinating dish
and ascertain the average measurements of the root-hairs and

[1] Ayers, H.   Methods of study of the Myxamoebae and the plasmodia of the My-
cetozoa.   Jour. f. Applied Microscopy, 1 : 1.   1898.

their frequency on the roots.   Compute the number per square millimeter.   Grow a second lot in water cultures.   Compare the features of the root-hairs mentioned above.

Collect living specimens of *Ranunculus delphinifolius* Torr. (*Ranunculus multifidus* L.).   Note that the long stems carry two forms of leaves.   Those under the surface of the water are divided into numerous capillary segments, while those in the air are divided into three to five broad lobes.   Examine the structure of both kinds by means of cross sections.   Describe the charac- ter of the epidermis with especial attention to stomata in both in- stances.   If possible grow specimens in water and note the man- ner of formation of the two forms of leaves.   Similar observations may be made on *Sagittaria*.[1]

[1] Schenck, H.   Vergleichende Anatomie der submersen Gewächse.   Bibl. Bot. 1 : 1.   1886.

V. RELATION OF PLANTS TO GRAVITATION

109. Nature of the Relation of Gravitation to Plants. All of the agencies besides gravitation, which affect protoplasm, or irritate it in any manner, consist of some form of chemical or physical energy which may affect the molecular motions of living matter directly. Gravitation is a force however, which has no direct effect upon protoplasm, although it causes well-defined reactions.

The emergence of the primitive ancestors of the higher plants from aquatic habitats brought the vegetal organism into a new set of conditions both for nutrition and distribution of reproductive bodies. As a result of these conditions, and also of the fierce competition arising from the enormous multiplication of individuals, and consequent increase of the number of species, it became highly advantageous for the plant to hold its shoot in an upright position above the substratum, and to send the roots or absorbing organs in the opposite direction. A sensory organization for the maintenance of such positions finds only one constant force by which it may be guided, which is gravitation. The irritability to gravity which has been acquired by the plant is therefore one of association, and not one of direct causal adaptability.

Beside the principal reactions by which the body is placed parallel to the line of action of gravitation, differentiations of this form of irritability enable the branches and secondary organs, leaves, etc., to assume a position at, or near, a right angle to the line of action of gravity.

Experimental tests of the action of gravity upon protoplasts by means of centrifugal force show that abnormally intense gravitational forces merely cause mechanical disarrangement of the organs of the cell according to their weight and density, and since such experiences do not accrue to the plant, no adaptive re-

action is shown, although it is supposed that this displacement may form the method of perception of the stimulus in sensory zones.   Gravity is found to exert a slight influence upon the rate of growth of complex organs, as a mechanical consequence of weight, which may also set up stresses with their own proper results, in compression and stretching reactions.

As will be shown below, gravity exerts some influence upon the disposition, origin and form of organs.[1]

110. Nature of the Stimulating Influence of the Force of Gravity.   No actual demonstration of the manner in which gravity acts upon an organ as a stimulus has yet been made.   The two principal theories suggest that the gross weight of the plastic organ acts as a stimulus, by its change of form when thrown out of equilibrium with gravity, or that bodies like centrosomes,[2] the heavier organs of the cell or the granules in the starch sheath act in the same manner as the heavy bodies in a compound statolith.   In the last named method the granules would fall to the lower side of the cell and their contact with the ectoplasmic layer would give the actual stimulus.   Neither of these methods would account for all of the features of geotropic reaction however.[3]
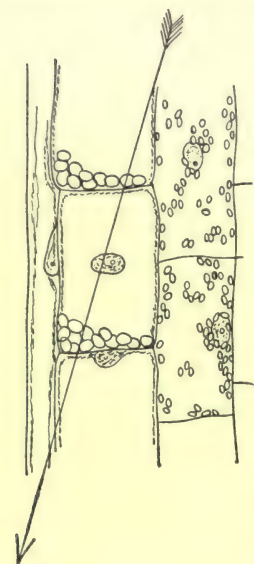
FIG. 30.   Portion of a longitudinal section through a node of *Tradescantia Virginica*, the long axis of which forms an angle of 13° with the vertical.   The arrow marks the direction of the action of gravity which throws the starch grains to the lower left-hand angles of the cells in the sheath.   After Haberlandt.

[1] Mottier, D.   The effect of centrifugal force on the cell.   Annals of Botany, **13** : 325.   1899.

[2] Czapek, F.   Weitere Beiträge zur Kenntniss der geotropischen Reizbewegungen. Jahrb. Wiss. Bot. **32** : 175.   1898.

[3] Noll, F.   Ueber heterogene Induktion.   1892.

Noll, F.   Ueber Geotropismus.   Jahrb. Wiss. Bot. **34** : 457.   1900.

111. Latent Period, Reaction Time, Presentation Period. It is a familiar observation that a stem of almost any ordinary plant which grows upright habitually, will show a curvature of the tip if the plant is laid on its side for an hour. The period elapsing between the prostration of the stem, which allows the geotropical stimulus to act, and the beginning of the upward curvature, is the *reaction time*, or *latent period*. Not all of this period is necessary to secure the curvature however. If the plant is laid on its side for 15 to 20 minutes and then set upright, or revolved on its own
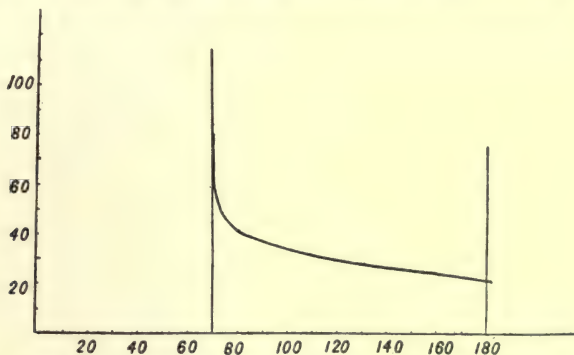
FIG. 31. Curve of geotropic reaction showing relation of exposition time and re-action time. The figures along the vertical line indicate exposition times, and those along the horizontal line reaction times. After Czapek.

axis in a horizontal position, the curvature will take place. The conclusion is justifiable therefore, that a portion of the original period was necessary for the reception of the stimulus, and that the remainder was necessary for the action of the curving mechanism. The first period is termed the *presentation time* and is included in the reaction time. The action of gravity during the presentation time may be likened to a summation of numerous stimuli, the effect of which cumulates with their repetition. Thus most hypocotyls and roots show curvature after being placed in a

Němec, B. Ueber die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen. Ber. Deut. Bot. Ges. **18**: 241. 1900.

Haberlandt, G. Ueber die Perception des geotropischen Reizes. Ber. Deut. Bot. Ges. **18**: 261. 1900.

horizontal position for 60 to 80 minutes.   If placed in this posi-
tion for a less time, then revolved on a clinostat the relation of
the length of the reaction time to the duration of the stimulus
(exposition time) may be found.

The rotation of an organ on its own axis, in a position in
which it receives geotropic stimulation presents all sides of the
sensory zone to the action of the stimulus, and thus brings the
organ into a state of tetanus so well balanced that no curvature
is shown.   If gravity is replaced by centrifugal force, or rather,
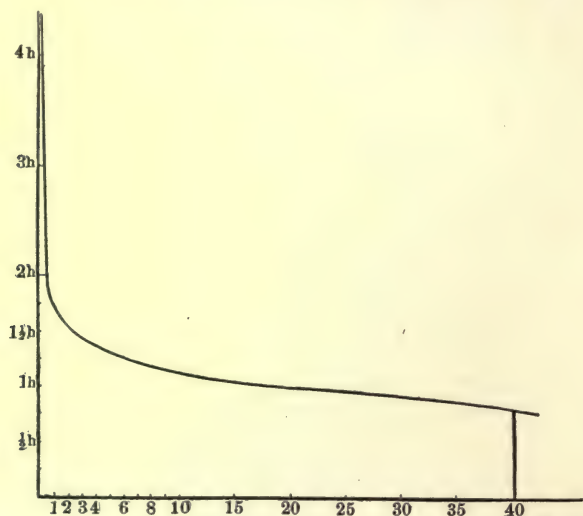
FIG. 32.   Curve showing relation of amount of centrifugal force and reaction time
of roots of *Lupinus albus*.   Reaction times are denoted by the figures along the verti-
cal line, and amount of centrifugal force in terms of gravity along the horizontal line.
After Czapek.

counteracted by it by being given in a different plane, an amount
of the centrifugal force amounting to but a thousandth of gravity
may be followed by a reaction after a period of six hours, and
when the centrifugal force is increased to 40 g. the reaction time
is shortened to 45 minutes.

The angle of divergence from the normal geotonic position
of the organ exerts such effect that the maximum stimulus is re-

ceived by orthotropic organs at a divergence of 135° from the
normal, and plagiotropic organs at 90° from the normal.[1]

In the following experiments the term *progeotropism* will be
used to denote the form of reaction which places the apex of
the organ in a vertical position
and pointing toward the center
of the earth. *Apogeotropism*
designates the reverse action,
and *diageotopism* denotes the
action of organs which place
their axes at right angles to the
line of gravity (See also lateral
geotropism).

**112. Determination of Reac-
tion Time.** Place a number of
seeds of *Avena sativa, Phalaris,*
or *Beta vulgaris* in a moist cham-
ber, or in damp sawdust until
germination takes place. Fasten
these seedlings in a plate of
cork by means of a pin driven
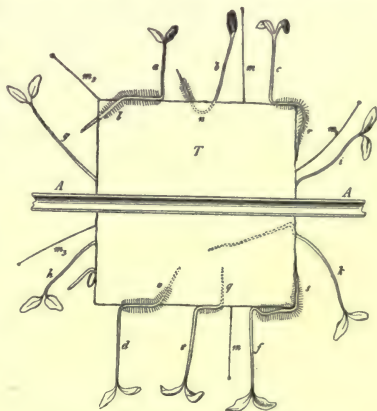
FIG. 33. Plate of cork or cube of turf
attached to extension arm of clinostat.
Seedlings are fastened to the cork or turf
and the original positions of the shoots
and roots marked by pins.   After Sachs.

at an acute angle through their main axes, with the tips of the
roots directed upward at an angle of 135°, and the hypocotyls
downward at the same angle.   Float the cork in a shallow dish,
and cover with a bell-jar in such manner that a horizontal micro-
scope may be focussed on the tip of a root.   During the first
few minutes a downward sagging movement due to the weight
of the plastic roots may be observed, and should not be confused
with irritable reactions.   Adjust the instrument to take up this
motion, and keep the tip of the root on the cross-hair of the
instrument.   Twenty-five minutes later keep under continuous
observation and note exact moment when downward movement
begins.   The temperature should be maintained at 18 to 25° C.

[1] Czapek, F.   Weitere Beiträge zur Kenntniss der geotropischen Reizbewegun-
gen.   Jahrb. Wiss. Bot. **32:** **175.**   1898.

Repeat with a seedling in a horizontal position so that the axis will be at an angle of 90° with the horizontal. Repeat with the root and hypocotyl only 45° from their normal vertical position. Compare the data obtained in the set of three tests.

113. Determination of the Presentation Time. Place a clinostat in position in a room with a temperature at about 20° C. and adjust the extension axis in a horizontal position, and attach a moist chamber. Fasten several seedlings in the chamber with their axes in a horizontal position, and mark the exact position of the apices of roots and shoots by means of additional pins thrust in the cork plate to which they are attached. Allow the apparatus to remain at rest for fifteen minutes, which will give
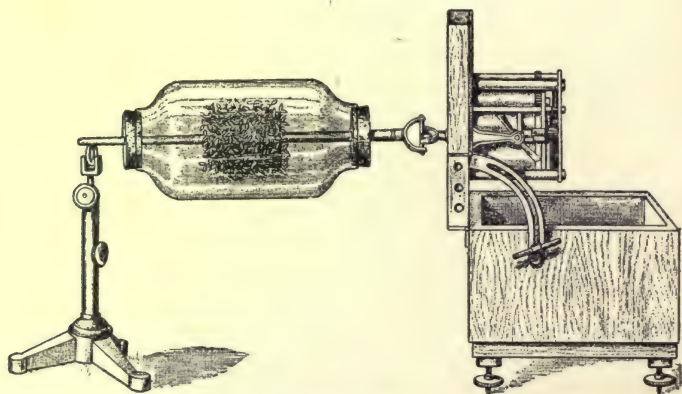
FIG. 34. Clinostat with moist chamber, containing seedlings, on extension arm. After Giesenhagen.

the roots and shoots a geotropic stimulus, but will not comprise the full reaction time. Set the clinostat in motion at such rate that it will make one revolution in ten minutes or slightly less. After three hours stop the apparatus and note the position of the apices in order to detect curvatures. Repeat the experiment, allowing the seeds to remain at rest to receive the stimulus for 30 and again 50 minutes before setting the apparatus in motion.

114. Effect of Slow Revolution of the Clinostat. Secure a seedling of a species which has been tested as above, and put it in a cylindrical glass moist chamber and fasten it to a piece of cork by means of a pin. Cover the chamber with a cloth to exclude the light, or perform the test in a dark chamber. Fasten the glass cylinder to the horizontal arm of the clinostat so that it may revolve about its own axis. Adjust the instrument so that it will turn the main shaft through one revolution in about twice the reaction time of the seedling. Note an hour or two later. Generally no curvature will be seen. As the root revolves about its own axis it is stimulated successively upon all sides, but not sufficiently to secure a perception or presentation of the stimulus. Now lengthen the period of revolution of the clinostat to four or five times that of the presentation period. The roots will be seen to curve toward all sides of the chamber in succession, in response to the several geotropic stimuli which it has received.

115. Influence of External Conditions upon Geotropic Reactions. Changes in temperatures between 15 and 30° C. cause only minor variations in geotropic reactions. Above and below these figures the presentation time is greatly lengthened.

Anaesthetics even in minute proportions decrease the delicacy of the sensory action, increasing both the presentation and reaction times. Carbon dioxide, or any inert gas that excludes oxygen, hinders geotropic reactions, and other chemical agents exercise an effect due to the manner in which they promote or decrease general metabolic activity.

116. Delayed Reactions. If an organ is placed in a position to receive a geotropic stimulus, and is held mechanically so that the reaction movement may not take place, for a period of several hours, the removal of the mechanical hindrance may result in a curvature even though the zone of growth has moved forward and the curvature is caused by a different lot of protoplasts from that which was originally stimulated.

117. After-Effects of Stimulation. The after-effects of a stimulus which has been given an organ, and response prevented

temporarily may be demonstrated in stems of grasses. Cut
a length of 12 cm. from a grass stem which will comprise
several internodes. Fasten it to a sheet of cork by means of
pins in the form of an X at both ends, and a third pair within
5 cm. of the basal end to give steadiness in the latter part of
the test. Place the cork in a horizontal position in a moist cham-
ber, or float in a dish of water and cover with a bell-jar. After
three or four hours remove the pins that fasten the apical end of
the stem and note result. Measure the angle of curvature.
Bend a stem of a grass over and pin it to a sheet of cork,
or run it through a glass tube just large enough to receive it,
and fasten the preparation in a horizontal position. Maintain
proper cultural conditions and examine two weeks later.
The excitation of the stem by gravity will set the mechanism for
curvature in action but the stem can not bend. The altered
growth following curvature takes place however, and forms en-
largements of the pulvinus, growing out into bulging ex-
crescences.

118. Sensory Zone of Roots. The cells which have the capacity
of receiving the geotropic stimulus in the root appear to lie within
a millimeter of the tip and embrace the region of undifferentiated
tissue. Two lines of experimental evidence tend to this con-
clusion. When this portion of the root is cut away the organ
ceases to be irritable until regeneration takes place. This test
offers insufficient evidence since the shock of the wound might
inhibit the normal reaction. A second experiment, exploited by
Pfeffer and extended by Czapek is free from errors of this sort
and is quite conclusive. The essential part of this demonstration
consists in forcing a root to grow into a small section of tubing
bent at right angles. When a portion of the root-tip a milli-
meter in length passes into the tube and is forced to take a posi-
tion at right angles to the main axis, it is found that the geotropic
sensibility of the root is determined wholly by the position of
this terminal portion. If the terminal portion is allowed to re-
main in the tube in a horizontal position it will elongate until it

acquires the power of curvature, then set up a reaction to direct the tip vertically.[1]

119. Alternating and Intermittent Stimulation.   It is found that an intensity or duration of the geotropic stimulus not sufficient to call out a reaction alone, may do so, if repeated at regular intervals for a sufficient number of times.   One method of accomplishing this demonstration consists in placing the stem of a grass in a moist chamber and revolving it on its axis in a horizontal position.   The clinostat is so arranged however that the stem remains motionless for a regular length of time and is then revolved an equal period, then is held motionless, etc.   After a

FIG. 35.   Showing method of cutting off shoots under water.

time curvature is produced as a result of the summation of the effects.

Most interesting results have been obtained by F. Darwin and D. Pertz by giving a stem two sets of diametrically opposed stimuli.   The clinostat was arranged to make half revolutions with periods of rest so that the stem bent first in one direction then in another.   As the result of this alternating stimulation the stem acquired a regular rhythm of movement which continued

[1] Czapek, F.   Ueber den Nachweis der geotropischen Sensibilität der Wurzelspitze. Jahrb. Wiss. Bot. **35** : 313.   1900.

for a short time even after the stem had been brought to a complete rest, and placed in an upright position.[1] Similar results have been obtained with phototropic stimuli by Czapek and Wiesner.

120. Rhythmic Effects of Alternating Stimulation. Select a small vigorously growing shoot of some convenient plant such as *Helianthus*. Cut off the tip under water and pass the base of the excised part through the cork of a bottle filled with water. Fix a fiber or small needle to the tip in a position exactly parallel to the axis of the shoot. The shoot must be about 12 to 20 cm. in length. Place the bottle on its side with the shoot in a horizontal position. Set a finely divided scale in a perpendicular position to one side of the needle, or fiber index. As soon as the index begins to travel upward, showing that the shoot is curving apogeotropically, revolve the bottle half way round on its axis. Note the position of the point and follow its movements for an hour or two. Observe again to 14 hours later.

121. Chemical Changes in a Geotropically Stimulated Root. Place a number of seedlings of *Vicia Faba* in a damp chamber for an hour with the roots horizontal, and others with the roots directed normally downward. An hour later select one root of each lot and quickly slice into four longitudinal sections. Lay the sections in watch crystals containing an emulsion of guiacum in ten-per-cent. alcohol, which has been prepared some time before. The sections from the unstimulated root are seen to take a darker blue color than the others.

Crush a number of stimulated and unstimulated roots in separate watch glasses full of salt solution consisting of 1 g. sodium chloride in 1000 cc. of water. Add a few drops of sodium hydrate and set aside for an hour. The preparation from the unstimulated organs will show a darker brown color than the other. The lack of color in the first and its presence in the second, is taken by Czapek to be due to the presence of an oxidizing ferment in the

[1] Darwin and Pertz. On the artificial production of rhythm in plants. Annals of Botany, **6** : 245. 1892.

unstimulated root, which is destroyed during the presentation period (See oxidases).[1]

122. Transmission of Geotropic Impulses. The region in which the curvature of roots ensues lies within 2 mm. of the apex of the root and is but slightly separated from the sensory zone. The nature of geotropic impulses is not understood. The path of conduction probably lies along the inner half of the periblem cylinder, and the fibrillae of Nĕ-mec may serve as organs of con-duction through the cells (14).

The conclusions of Nĕmec on this subject have not been tested by additional investiga-tions at this time. It is known however, that transmission in roots is influenced directly by all agents which modify the motility of protoplasm, such as chloro-form and other anaesthetics, and the method of conduction is therefore different from that ex-hibited by *Mimosa*, and much less rapid. An impulse set up by a shock in *Mimosa* travels about 600 to 1,000 times as fast as the geotropic impulse of a root.
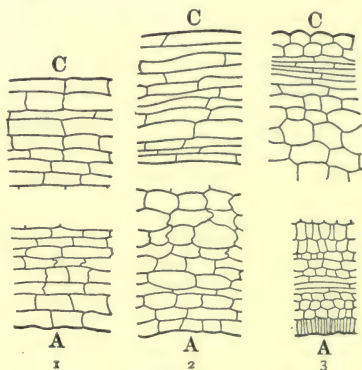
FIG. 36. Changes in tissues of roots during curvature. 1, sections from curved portion of root of *Zea mais* three hours after excitation began. 2, same 20 hours after excitation began. 3, sections from curved portion of root of *Phoenix dac-tylifera* 20 hours after beginning of ex-citation. *A, A, A,* epidermis of con-cave side. *B, B, B,* epidermis of con-vex side.

123. Mechanism of Curvature of Roots. Germinate a number of peas, beans, or seeds of corn in damp sawdust. When the radicles have attained a length of 3 or 4 cm. fix them in saw-dust, or place in position in damp chamber with the tips pointing nearly upward. Examine at intervals, and as soon as a root shows a curvature of more than 45°, cut away the curved por-tion with a sharp razor and fix in acetic alcohol, or Flemming's

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[1] Czapek, F.   Ueber ein Befund an geotropisch gereizten Wurzeln.   Ber. Deut. Bot. Ges.   **15 : 516.**   1898.

7

solution of osmic, acetic and chromic acid, dehydrate, wash, in-
filtrate and imbed in paraffine, a process that takes a few days.
Cut the root into a series of longitudinal sections, using an auto-
matic microtome and fasten to glass slips.[1] Dissolve out the
paraffine and bring the material by proper stages through mix-
tures of alcohol and xylol into 40 per-cent. solutions of alcohol.
Stain in a solution of Bismark brown in 40-per-cent. alcohol and
return by proper stages to pure xylol and mount in Canada bal-
sam.   Now measure the distance from the tip to the region of
curvature.   Compare the cells of the tissues in correspondent
positions on the convex and concave sides of the organ.   Meas-
ure carefully and note staining reaction on the walls and ecto-
plasm.   What differences in quality, size and contour are to be
seen ?   Make similar sections through a straight root and com-
pare size and form of cells.[2]

124. Diageotropism of Secondary Roots.   Germinate some
seeds of the pea or bean in moist sawdust until a number of sec-
ondary roots have been formed.   Now remove the seedlings and
place in a moist chamber with the main root horizontal, or put
in the same position in the sawdust.   Either preparation should
be kept in the dark room.   Two days later note the position of
the secondary roots.   These organs will be found to occupy a
position departing more or less from the horizontal and constitut-
ing diageotropism.

125. Sensory Zones of Shoots.   The entire growing region of the
shoot of many seed plants may be capable of appreciating geotropic
stimuli, and it is not possible to make such sharp delimitation as in
the case of the sensory zones of roots. The sensory function may
be more highly developed in some tissues than in others, generally
reaching its greatest delicacy in the cortical cells, though the
pith is capable of both perception and reaction in some species.

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[1] Zimmerman.   Botanical Microtechnique.   1893.

[2] MacDougal.   Curvature of roots.   Bot. Gazette, **23** : 340.   1897.

See also Pollock, J. B.   The mechanism of root curvature.   Bot. Gazette, **29** : 1.
1900.

126. Region and Form of Curvature of Shoots. Grow a number of seedlings of *Helianthus.* When a few centimeters high take them from the sawdust or sand in which they have been grown, and fix a half dozen by means of split corks in bottles of water. Mark the stem of each into centimeter intervals by means of a ruler, and a thread saturated with India ink and held taut by a pair of calipers. Now lay the bottles on their sides in a damp chamber at a temperature of about 20° C. in diffuse light for three hours. Note the region in which curvature occurs. Allow the curvatures to proceed. It will be seen that the tip of the shoot will be carried upward and past the vertical and then back again (See Fig. 37).

127. Mechanism of Curvature of Grass Stems. Cut a few rapidly growing stems of some convenient grass and mark 2 mm. intervals on opposite sides of some of the nodes. Now place these stems in a horizontal position, with one end in a heap of moist sand and cover the whole with a glass vessel to make a moist chamber. Take out the stems a day later and measure the distance between the marks on the upper and lower surfaces of the nodes. Note that the hairs on the lower surface are diverg-
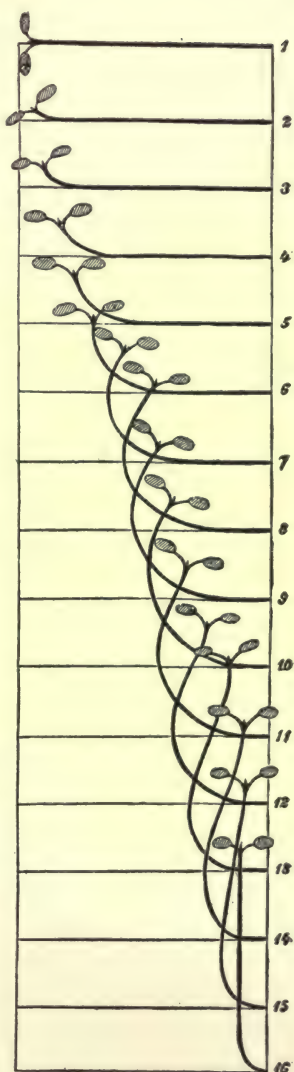
FIG. 37. Apogeotropic curvature of a shoot. 1, horizontal position of stem at beginning of experiment. 2-15, successive stages in upward curvature. 16, final position of shoot. After Sachs.

ing while those on the upper are convergent. Examine for differences in structure.

128. The Geotropic Relations of Dorsiventral Organs. The greater number of dorsiventral organs, such as leaves, are dia-

FIG. 38. *A*, stem and branches of a pine tree 7.5 meters in height from which the top has been removed. *B*, same a year later showing upward curvature of a branch seven years old. After Jost.

geotropic, although many well-known exceptions occur. Such organs are in equilibrium only when the axis subtends a certain fixed angle with the line of gravity, and when the two sides occupy their proper and relative positions. The geotropic reactions of such organs may show two movements : a curvature to bring the axis toward the horizontal, and a twisting or torsion to place the surfaces in their normal relative positions. These reactions are generally complicated with phototropic movements in plants growing in the open, and are not capable of easy analysis.

129. Rotation and Curvature of Petioles of Dorsiventral Leaves in Response to Geotropic Stimuli. Secure a vigorous specimen of any species of *Helianthus* growing in a pot. Place the axis of the plant in a horizontal position in a dark room and note the positions of the leaves with respect to the stem, and ascertain what curvatures and torsions have taken place.

130. Geotropic Curvatures in Organs in which Growth in Length has Ceased. It has been a matter of common observation that the branches of many trees, two, three or more years old, undergo upward curvatures. Such curvatures have been observed in branches of many conifers 4.5 cm. in thickness and progeotropic curvatures in the weeping branches of many trees have also been seen. Jost records the apogeotropic curvature of a branch of a fir seven years old[1] (Fig. 38).

[1] Vöchting, H.   Organbildung im Pflanzenreich. **2** : 85.   1884.

Jost, L.   Ueber einige Eigenthümlichkeiten des Cambiums der Bäume. Bot. Zeitung, **59** : 1.   1901.

The curvature here must be ascribed to the activity of the cambium of the lower side of the branch, which becomes the convex surface of the organ.

131. Diageotropism of Flowers of Narcissus. Cut off some stalks of *Narcissus poeticus* the flower buds of which have just opened and place some in a horizontal position, and others in a vertical position. Or simply lay a pot containing the plants on the side. Either preparation should be placed to receive all of the light reaching it, from one direction only. Note the movement of the peduncles. These curve to place the calyx tubes in a horizontal position. What direction do they take with respect to the light? Note torsions as in last experiment (See carpotropism).

The geotropic stimulus induces the curvature toward the horizontal position, but it will be found that the direction in which this curvature is made will be determined by the rays of light, the tubes pointing toward the source of the rays. Observe the behavior of a specimen placed in a dark chamber.

132. Lateral Geotropism of Twining Plants. A large number of species of plants have acquired the habit of lifting their leaves into the sunlight by twining their slender stems around other plants, or any convenient support. In order to accomplish this it has been necessary for them to develop a special form of reaction to gravity, which may be termed *lateral geotropism*. By this form of irritability the growing tip of the inclined stem of a twiner is stimulated to curve horizontally. The curving portion is directly connected with the firmer internodes below, and the flexion is necessarily accompanied by a rotation of the axis of the apical portion of the stem thus exposing a new region to the stimulus of gravity and setting up a new curvature, which causes the stem to be stimulated to curve in a slightly different plane, and this repeated change of position and rotation induces a continuously altered geotropic response that sweeps the tip around in an irregular circular manner. This action may be imitated if a section of heavy rubber tubing a few cm. in length is held in the

hand and the tips made to sweep around in a circle. This motion results in winding the twining stem around a support. After a time such stems generally lose their lateral geotropism and become weakly apogeotropic, which binds them more closely to the support.

133. Revolving Movement of Tips of Twining Plants.   Select a strong plant of the bean, hop, morning glory, or other twining species and fasten the fully grown part of the stem to an upright stake or support, which will allow the immature internodes with a total length of 8 to 20 mm. to project above its upper end.
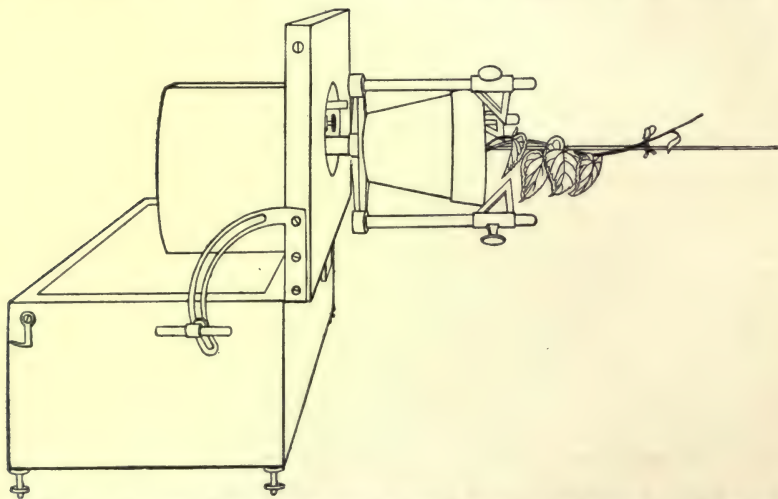
Fig. 39. Twining plant being rotated on its own axis by a clinostat.   The vine fails to clasp the support and must be held to it by cords.

Draw a line along one side from the tip of the shoot to the point of attachment to the stake with India ink.   Ten minutes after this has been done set up a small stake which shall mark the position of the tip, but which does not touch it or impede its free movement in any way.   Set up a second stake in 20 minutes and another at the end of every 20 minutes for an hour, or until the tip has traversed a circle.   Determine the region in which the curvatures are produced, and note the position of the ink line to

find torsions of the stem.[1]   Observe the movements of all of the
climbing plants to which access can be had and note direction in
which the revolving movement is made and time necessary for
one revolution.

**134. Behavior of Twining Plants when Freed from the Influence
of Gravity.**   Select a small specimen of one of the species used
in 133, not more than 25 cm. in length, growing in a suitable pot,
and tie the stem to a stake as before.   Now fasten the pot to a
clinostat and revolve it in a horizontal position on its long axis.
The tip of the shoot should be directed parallel to the surface of
a window, and the revolutions of the instrument should be made
in less than ten minutes.   Note that the stem ceases its revolving
motion and grows in a more or less direct line, when freed from
the unequal lateral stimulation of gravity (Fig. 39).

135. Alterations in Geotropic Properties.   Vegetative organs
generally retain their characteristic geotropic properties during
their entire existence unchanged.   In some instances however,
the form of reaction exhibited may be reversed by the influence
of external agents, such as oxygen, or the lack of a proper sup-
ply of this element, or by the influence of the phototropic prop-
erties of the plant.   The reproductive shoots of the higher
forms may exhibit direct alteration of geotropic properties cor-
responding to the changed needs of the organism in accomplish-
ing pollination, seed-distribution, and protection from climatic
elements.   Thus the buds and flowers of *Papaver*, flowers and
fruits of *Aquilegia, Delphinium*, and *Aconitum* change their geo-
tropic reactions during the course of pollination, fertilization or
development of the seeds (See carpotropism).[2]

136. Recovery from a Position Assumed Geotropically.   If a
root or shoot has formed a curvature in response to a geotropic
stimulus, and is then set in such position that it will be stimulated
in a reverse manner the curved portion will first be straightened,

[1]Arthur.   Exp. in Veg. Physiol.   1897.
Darwin, C.   Climbing Plants.   1876.
[2]Vöchting.   Bewegungen der Blüten und Früchte.   1882.

and then recurved in the opposite direction. If however, the first position has been held for a length of time sufficient to permit of any notable growth or development of the tissues, the curvature will become fixed and permanent. The second curvature is generally slower than the first. Roots which need but two or three hours to attain a position of equilibrium in response to the first stimulation, will require 12 to 24 for the unbending and recurving in a new position. The above statement applies only to growing organs and not to pulvinar movements.

137. Recovery of Curvature of Roots. Place a root of pea or bean in an inverted position by fastening the seedling to a sheet of cork in a moist chamber for two or three hours, or until a full curvature has been accomplished. Now alter the position of the organ until the tip again points upward, so that it will reverse or straighten the curvature in order to reach a position of equilibrium with respect to gravity. Note time and manner of this second reaction.

138. Formative Influence of Gravity. Cut two willow, or poplar branches 25 cm. in length, and place in a moist chamber the atmosphere in which is kept completely saturated. Suspend one twig in a horizontal position, and the other in a vertical position with the apical end downward. The polarity of the twigs would induce them to form leaves from the apical portions of both twigs, and roots from the basal end. In response to this, such formation will occur at the beginning of the experiment. If the test is continued for a few weeks however, it will be seen that roots continue to develop from the lower side or end of the shoots, and leaves from the upper sides or ends (See correlations).

The above test may be performed most successfully if the twigs are taken in the spring immediately before breaking out of the buds. The moist chamber should be placed in diffuse light which should come from several directions, or the experiment may be carried on in the dark chamber.[1]

[1] Vöchting, H.   Ueber Organbildung im Pflanzenreich.   1884.

VI. RELATION OF PLANTS TO TEMPERATURE

139. General Relations of Temperature to Protoplasm. Temperature is one of the most widely interlocking factors concerned in the activity of protoplasm. This is due chiefly to the fact that the characteristic processes of living matter are essentially chemical changes, in which the molecular activity of the engaged elements as well as their physical properties are affected directly by the temperature. All absorptive and excretory functions, respiration, enzymatic action, synthetic processes, turgidity, germination, growth, reproduction and adaptive movements are directly dependent upon the temperature of the body of the plant, which follows more or less closely the medium in which it exists. Variations in temperature inside the limits of tonicity induce reactions expressed by altered metabolism, or movements, and continued existence in any given average allows it to become a distinctive formative factor. In addition it is also to be said that the radiant energy of heat waves forms a source of energy to the plant.

140. Tonicity to Temperature. A maximum and minimum may be distinguished for most plants. Between these lies a temperature at which the greatest activity is reached, constituting the optimum. The optimum shows great variation among dissimilar organisms. It varies from 26.6° C. to 37.7° C. among the seed plants, and from 20° C. to 70° C. in the lower forms inclusive of bacteria.

The maximum temperature of the higher plants ranges from 37° C. to 46° C. according to the species, individual, and stage of development. Among the simpler forms the maximum may be much higher, especially in bacteria and algae living in warm springs and thermal waters. At or above the maximum, protoplasm passes into a state of immobility known as heat rigor, from

which it may revive when brought into a lower one.   Above the temperature producing heat-rigor, is a point at which the condition of inactivity induced in heat rigor becomes one of death-rigor from which the organism may not recover.   Death rigor sets in at 46° C. with some of the higher plants, though higher than this in others.   The bodies of fleshy cacti have been found by the author to reach a temperature of 46° C. in the desert plains of Arizona.   Seeds and propagative organs have special adaptations for the endurance of much higher temperatures however, and spores of certain bacteria (anthrax spores) are reputed to live and develop after being subjected to 140° C.

The minimum at which activity may proceed varies with the same conditions that influence the determination of the maximum. It is, however, rarely below 0° C. and generally lies a few degrees above that point.

The discussion of the relations of temperatures below the minimum brings out some of the most interesting facts in the entire subject.   The degree of cold necessary to produce death-rigor varies with the experience of the organism, and its species, the rate of cooling of the organism, duration and exposure, and rapidity of rise in temperature at the close of the tests.   The amount of water in the protoplasts, and the stage of development are very important factors in such endurance.   −252° C. is the lowest temperature to which vegetal protoplasm has been exposed, and this condition was obtained by immersing seeds in liquid hydrogen, in which they remained for an hour. *Brassica alba, Pisum sativum, Mimulus Moschatus, Cucurbita Pepo, Triticum sativum,* and *Hordeum vulgare* are represented in this result. [1]

It is to be seen that protoplasm has taken such adaptive forms in different plants that it may withstand a total range of 392° C. (705.6° F.), which is far beyond the capacity of but few single organisms, however.

[1] Thiselton-Dyer, W. T.   On the influence of the temperature of liquid hydrogen on the germinative power of seeds.   Proc. Roy. Soc. **65** : 361.   1899.

TABLE SHOWING CRITICAL POINTS IN TEMPERATURE OF
REPRESENTATIVE SPECIES. [1]

	Minimum.	Optimum.	Maximum.	Remarks.
Zea mais (plumule)	9.5° C.	33.7° C.	46.2° C.	
Zea mais (radicle)		34		
Phaseolus multiflorus (plumule)	9.5	33.7	46.2	
Phaseolus multiflorus (radicle)	.	26.3		
Sinapis alba (plumule)		27.4	37.2	
Hordeum vulgare (plumule)	5	28.7	37.7	Endured —252° C. in seeds.
Yeast	0	28–34	38	Endured —113.7 and killed by 53.
Pencillium	1.5	22	43	
Bacillus phosphorescens	0	20	37	
Bacillus tuberculosis	30	38	42	
Bacillus thermophilus	42	63–70	72	

141. Adjustment to Changes in Temperature. The establishment of a plant at any temperature, by enclosing it in a medium, is not followed immediately by the activity characteristic of that temperature ; some time is necessary to call up the effect. Thus if an organism is at the minimum temperature, and is raised to the optimum, it will be some hours before the beneficial change will be followed by the usual rate of growth. If the temperature of the medium be raised or lowered gradually it is possible for many organisms to become acclimatized, which entails the adjustment of the three critical points. Such acclimatization must consist chiefly in changes in the proteid bodies in protoplasm, and be connected with variations in the amount of water of constitution present.

142. Stimulating Influence of Changes in Temperature. Sudden changes in the intensity of the heat rays, or of the temperature of a plant constitute a stimulus which brings its own proper response. These reactions may be due directly to the varied chemical activity of the compounds in the protoplasm, or may be adaptive responses on the part of living matter.

[1] Data taken chiefly from Davenport's Exp. Morphology, 1 : 219.   1897.

One of the most interesting manifestations of such irritability
is that shown by many seeds and propagating bodies, tubers, and
spores of plants living in high latitudes. Many of these forma-
tions may not be induced to emerge from the resting period until
they have undergone a period of low temperature, in imitation
of the winter through which they naturally pass. The shock of
change from a law to a high temperature seems to be necessary
to start the protoplasmic machinery in action, and may perhaps
serve as an indirect signal stimulus.

143. Resistance and Acclimatization of Seeds to Heat. Secure
two or three hundred seeds of pea, radish or corn, by selecting
only those apparently capable
of germination. Place a dozen
in a Zurich germinating dish
and note time necessary for
germination, and proportion of
active seeds. Place 100 seeds
in an incubator the tempera-
ture of which is under accurate
control and gradually raise the
temperature to 40° C. at which
point it should be maintained
for 12 hours. Take a dozen
seeds from the incubator and
germinate as above. Slowly
raise the temperature of the
incubator to 50° C. and place
in it an additional lot of seeds
not previously heated. Main-
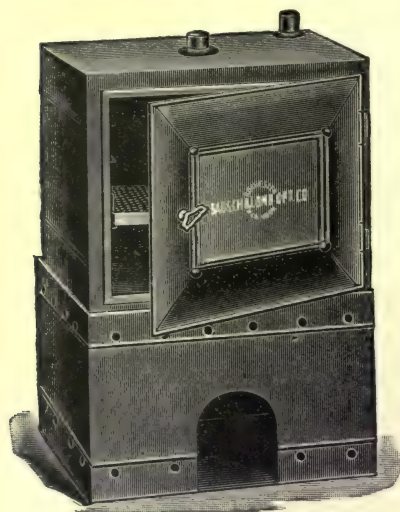
FIG. 40. Form of incubator suitable for
tests of endurance of temperature by seeds

tain the temperature for 12 hours. Put the freshly treated lot,
and a dozen of the others in a germinator. Note results as be-
fore. Raise the temperature of the incubator to 60° C., again
placing in it a fresh lot of untreated seeds, with the old lot. After
12 hours take out the fresh lot and a dozen others and germinate
as before. Repeat at 65° C., 70° C., 75° C. and 80° C. Tabu-

late the data thus obtained and plot results of observations on the following points : 1. Percentage of germinations of the continuously heated seeds at the different temperatures. 2. Percentage of germinations of freshly treated seeds. 3. Comparison with first control lot. 4. Fatal temperature of the untreated seeds. 5. Fatal temperature of the acclimatized seeds.

144. Relation of Water Content to Endurance of High Temperature. Select 100 plump seeds of corn or wheat. Plant ten in a germinator and grow. Place 50 in a small dish of water and after 12 hours set in incubator at 30° C. for 12 hours. Place 12 in germinator. Raise temperature to 40° C. for 12 hours and put in ten untreated seeds. Take out fresh lot and ten old seeds and germinate. Repeat at 45° C. and 50° C. Tabulate results as in

Fig. 41. Stage for exposure of mounted objects to different temperatures. *A, A'*, screws for clamping to stage of microscope. *B*, outlet tube. *B'*, inlet connecting with vessel containing water heated by a flame. *C*, condenser to illuminate object. A thermometer is set horizontally in the stage near the outer edge.

the previous experiment, and determine same points, noting also in addition the lessened resistance of saturated seeds. Compare the acclimatization results.[1]

145. Influence of Temperature upon Movement of Protoplasm. Mount a hair of *Tradescantia* or some convenient cell on a glass slip and place on a Reichert warm stage, or some other convenient form of apparatus on the stage of a microscope, and measure rate of movement of granules in a strand of cytoplasm by means

[1] Just, L. Ueber die Einwirkung höheren Temperaturen auf die Erhaltung der Keimfähigkeit der Samen. Cohn's Beit. z. Biol. d. Pflanzen. **2** : 311. 1877. See also, Kindsel, W., in Landw. Versuchssta., **54** : 134. 1900.

of an eye-piece micrometer.   Connect the inlet and outlet pipes properly and arrange a vessel to be heated by a gas flame or alcohol lamp for furnishing warm water.   This should be placed above the level of the stage and should be controlled by a pinch-cock which will regulate the amount of water flowing through the warm stage.   Raise the temperature of the slide to 37° C.   Compare rapidity of movement with that of previous temperature. Open the stopcock and allow a rapid flow of warm water, raising the temperature to 42° C.   Note results.   Raise the temperature by accessions of 5° C. until movement ceases.   Determine the point of heat rigor, from which the cell may recover and resume motion.   Determine the point of death rigor and note behavior of protoplasm.   The slip lying on the warm stage will be one or two degrees colder than the reading of the thermometer provided for the stage.   This error must be calibrated and taken into account in all readings.[1]

Mount a fresh object and place melting ice in the supply vessel taking away the lamp.   Lower the temperature to 25° C., 20° C. 18° C., 16° C., and lower by intervals of 2° noting minimum and fatal temperature producing cold rigor and death.   Expose fresh material suddenly to each of the above temperatures to determine whether acclimatization has taken place in the previous tests.

146. Relation of Low Temperatures to Resting Period of Bulbs and Tubers.   Secure two dozen hardy bulbs or tubers of potato or *Arisaema*, or some hardy plant.   Bury half of the lot in the soil out-of-doors and allow them to remain where they will receive the prevalent out-of-door temperatures until December 1st. Imbed the remainder in sawdust and set in dark corner of greenhouse or laboratory where the temperature does not fall to 40° C.   The first lot may be placed in a refrigerator in which provisions are kept instead of being buried in the soil from September to December.   Now place both lots in pots using proper methods of culture and set in temperate room with temperature between 60° and 65° F.   Note the behavior of the two lots.

[1] Beal, W. J.   *Bromus secalinus* germinating on ice.   Bot. Gazette, **23** : 204. 1897.

One has received the customary low temperatures during the resting period, and the other has been kept abnormally warm. The best results will be secured with some species native to the region in which the test is performed.

147. Freezing of Unicellular Organisms.   Mount a number of healthy filaments of *Spirogyra* on a glass slip and make examinations, and exact drawings of a few cells.   If the test is made in midwinter the slide may be placed outside on the window sill for half an hour, and allowed to freeze.   If made at other times or places, freezing mixtures of ice and salt may be employed, or still better the slide may be subjected to the action of escaping liquid carbonic acid, which will give instant and low temperatures.   Bring the slide frozen by any of these methods into the laboratory, and allow it to thaw gradually.   Keep under constant examination, and determine whether ice crystals are actually formed inside the cells or not. Note effects on cell and draw.

FIG. 42.   *Spirogyra. a*, normal. *b*, frozen and imbedded in ice. *c*, same after thawing.   After Molisch.

148. The Freezing of Tissues. Mount a leaf of *Philotria* on a glass slip and expose to freezing temperatures as above, and note results.   Repeat using sections of stems containing living parenchymatous and embryonic tissues.[1]

149. General Observations on Freezing.   The actual shock to a protoplast by freezing appears to be accompanied by the with-

[1] Molisch, H. Untersuchungen ueber das Erfrieren der Pflanzen.   Jena.   1897.

drawal of the water of constitution from the plasma and forma-
tion of it into crystals either inside or outside the cell wall.   In the
latter case the formation of intercellular ice crystals may often
result in tearing apart the cells of the tissues, but in no case has
it been found to rupture the walls except in very violent lowering
of the temperature.   Low temperatures even above the freezing
point may be fatal to an organism.   A few plants such as *Agave
Americana* may be frozen, and if the temperature is not carried
too low, may revive if thawed slowly.   In most instances how-
ever the rapidity of thawing from ordinary low temperatures is
without bearing upon the fatality of the process as also thawing
in air or water.   The rate at which the temperatures of plants
exposed to liquid air or liquid hydrogen ($-252°$ C.) is raised is
of importance on account of the great physical disturbances in-
volved.   Not all of the tissues of a plant are equally resistant to
cold.   The embryonic elements probably succumb most easily,
while the stomatal and trichome cells are most resistant in the
vegetative body.   The most effectual adaptations for the en-
durance of low temperatures are to be found in reproductive
bodies of all kinds.   Some very interesting observations may
be made upon the effects of low temperatures upon plants
if the student will spend the day following the first heavy
frosts in the field examining the native species for frost reactions.
A list of the species affected, and the organs killed should be
made.   It will be seen that perennial plants have varying pro-
portions of their bodies killed by frost.   In trees only the leaves
may die ; in shrubs and shrubby plants the shoot may die down
to the ground, and in certain herbaceous forms all but some
thickened fleshy roots may perish.

The topography of the region should also be taken into account
and the accumulation of cold air in valleys be followed and the
effects noted.

150. Formative Effect: Thermal Constants.   The formative ef-
fect of temperature is scarcely differentiated so far as its influence
upon individuals are concerned, although the general adaptations

of species living in alpine climates are very marked. The extremes of temperature are the tests for endurance of the protoplasts, but any species must receive a specific amount of heat or a certain amount of radiant energy in the form of heat in order to carry out the seasonal activity. It is this that determines the continued existence of a plant in any locality. These thermal constants are found by adding the daily maxima during the vegetative season of the species.[1]

151. Thermotropism. The free moving organisms of the animal kingdom show very marked movements in response to changes of temperature both as to movements of organs, and locomotion which will place their bodies in an optimum intensity of the radiations. This capacity is shared by free swimming spores of plants to some extent, while shoots, roots and secondary organs exhibit curvatures in some instances toward the source of heat, or to place their surfaces in such position as to decrease harmful radiations during periods of low temperature. The effects of changes of temperature are so intimately connected with the adaptive reactions to light, which are so regularly recurrent as to have become rhythmic, that it is difficult to distinguish purely thermotropic movements.

152. Thermotropism of Leaves. The leaves of a number of woody plants assume a drooping position at temperatures under the freezing point, and recover when the thermometer indicates a point much above that. This movement is shown by the laurel (*Prunus laurocerasus*), Portugal laurel (*Prunus Lusitanica*) and may also be seen in branches of *Tilia* during the first frost of the season.[2]

A very striking example of this action is shown by the great laurel, or rosebay (*Rhododendrum maximum*), which is found over the eastern United States. If specimens of this plant are examined after the temperature has fallen below the freezing point, it may be seen that the leaves are of a deep green color with a

[1] Kerner. Natural History of Plants. 1 : 558. 1890.
[2] Darwin and Acton. Physiology of Plants, 163. 1894.

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brownish hue, with the margins inrolled, and that the petioles
are curved sufficiently to allow the laminae to depend in a posi-
tion almost vertical.   Intense sunlight which does not raise the
temperature does not interfere with this action.   If now a branch
is cut from the plant and taken into a warm laboratory room at
20° C. to 25° C. the leaves will begin to rise within two minutes
and will have assumed a position nearly horizontal in five or six
minutes.   The base of the branch should be inserted in a vessel
of water as soon as brought into the room.   After the leaves
have come to a state of rest remove to the open air at a temper-
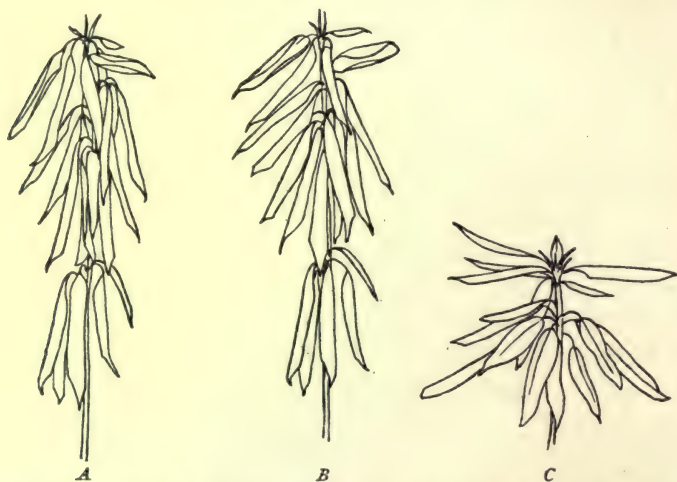
FIG. 43.   Branch of *Rhododendrum maximum* standing in vessel of water.   *A*,
showing position of leaves one minute after removal to warm room.   *B*, same, one mi-
nute later; upper movement of leaves has begun.   *C*, branch with leaves of a normal
warm position about five minutes after removal to warm room.   After Harshberger.

ature below zero C. and note the reverse movements, which will
be much slower.   Test the rigidity of the petioles when in a de-
pressed position.   The lax condition of the leaf suggests that the
reaction is due to lowered turgor in the cells on the upper side
of the leaf.   The general purpose of this reaction is doubtless the
same as that exhibited by leaves with pulvini, in which the leaf
is held in a rigid condition.   The drooping position of the laminae

would lessen danger from falling snow and ice and would also decrease transpiration and retard radiation of heat.[1]

153. Thermotropic Reactions of Shoots. Place a long table with one end toward a window, and set upright near the window a plate of sheet iron which has been smoked on the inner side by means of a candle flame. Place two or more gas jets back of the plate nearer the window in such position that the plate will be warmed over its whole surface. At the farther end of the table set a large mirror which will reflect light directly toward the plate. Adjust the ventilation and heat of the room to secure a temperature of about 12 or 13° C. Secure a number of seedlings of *Lepidium* in small pots. The seedlings should be about 3 or 4 cm. high and should be grown in two-inch pots. Set a pair of the seedlings at a point on the table where the air over the pot is at 35° C., and a second pair farther away where the temperature is 30° C. Maintain these temperatures for a period of four hours and note position of shoots in both pairs. The optimum temperature for *Lepidium* is about 33° C. and the shoots should tend to curve toward the source of radiation or away from it as they lie below or above the maximum.

Repeat the test with *Zea* seedlings two or three cm. in height, and set them in pairs at such distances as to secure temperatures of 30° C. and 25° C. over the middle of the pot. Several kinds of seedlings may be used at once, but it will be found that not all are thermotropic, either negatively or positively.[2]

154. Influence of Temperature on the Opening or Closing of Flowers. Select a cool cloudy morning and cut a flower of the tulip and fix the stalk in a bottle of water by means of a cork. Attach a fine filament or thread of glass to one of the outer perianth segments by cementing it with shellac to the groove on the outer surface of the organ. Fasten a similar filament to the opposite

[1] Harshberger, J. W. Thermotropic movement of the leaves of *Rhododendrum maximum* L. Proc. Acad. Nat. Sciences of Philadelphia. 219. 1899.

[2] Wortmann, J. Ueber den Einfluss der Strahlenden Wärme auf wachsende Pflanzentheile. Ber. Deut. Bot. Ges. 41 : 457, 473. 1883.

inner segment of the flower, allowing both filaments to project about three cm. beyond the flower. Set the bottle on a stand and adjust a millimeter scale horizontally so that the distance between the two filaments may be read off. Make the above preparations at a temperature of 12–15° C., and after a few minutes carry the preparation into a warm room at 20° C. Read the distance between the points of the filaments in 5, 10, and 15 minutes, on a horizontal millimeter scale.[1] Replace in a cold room or out of doors and note result.

155. Thermotropic Reactions of Tendrils, Dionaea, etc. The thermotropic reactions of tendrils discovered by the author,[2] and exploited by Correns,[3] as well as the movements of *Dionaea* when exposed to rapid changes in temperature, are examples in which the mechanism of response, designed to make adjustments to one class of forces, may be set in action by unlike agencies.

Such reactions may be observed if a tendril of *Passiflora* is quickly warmed eight or ten degrees by means of being thrust into a hot air chamber, or if a flask full of hot water is held near it. Thermotropic stimuli may be given *Dionaea* by thin streams of water at various temperatures.[4]

[1] Pfeffer, W.   Physiol. Untersuch. **181.** **1873.**
[2] MacDougal.   The tendrils of *Passiflora coerulea*.   Bot. Gazette. **18** : 125.   1893.
[3] Correns, C.   Zur Physiologie der Ranken.   Bot. Zeitung, **54** : 1.   1896.
[4] MacFarlane, J. M.   Contributions to the history of *Dionaea muscipula* Ellis. Contr. Bot. Lab. Univ. Penn. **1** : 20.   1892.

VII. RELATION OF PLANTS TO ELECTRICITY AND
OTHER FORMS OF ENERGY

156. Nature of Influence of Electricity upon Plants. The rela-
tions of electrical energy to plants are but imperfectly known, and
but few phenomena are capable of satisfactory demonstration. It
is well established that marked differences in electric potential are
found in all active plant bodies.   Such departures from a state of
equilibrium are due in part to the action of currents of water, and
also to the conversion of chemical and other forms of radiant
energy into electrical force in the metabolic processes.   Whether
the currents established in this way play any essential part in the
organism, or whether they represent total dissipations of energy
is not known.   It is quite probable however, that the movements
of fluids and gases are influenced profoundly by these currents.
Large plants, such as trees, with erect trunks extending upward
into the air, also serve as points of discharge of static electricity
between the soil and air and these discharges are often so intense
as to shatter the bodies of the plants.   Earth currents exercise a
directive influence upon the growth of roots, probably upon
other organs also.   Electrical energy exercises a very marked
stimulating influence upon protoplasm, inducing contractility.[1]

157. Measurement of Differences in Electric Potential. A cap-
illary electrometer, a key and a pair of non-polarizable electrodes
will be necessary to perform this experiment.   The electrometer
may be purchased from dealers in physical apparatus, and also
any simple key for opening and closing a circuit.   The electrodes
may be made as follows : Secure two small glass tubes a few cm.
in length and close one end of each with a plug of well-kneaded
modelling clay, through which projects a small camel's-hair brush.

[1] Stone, G. E.   Influence of electricity upon plants.   Bot. Gazette. **27** : **123**
1899.

Fill the tubes with zinc sulphate. The brushes should be arranged
so that the fluid will pass down through the quill handle and keep
the hairs moist. Close up the upper
end of the tubes with a cork stopper
through which passes a rod or strip of
zinc which has been amalgamated with
mercury. The wires should be soldered
or closely bound to the zincs. Connect
one of these electrodes directly to the
galvanometer, and the other through
the key. Test the apparatus by bring-
ing the electrodes in contact with one
another ; if no movement is shown by
the galvanometer it is correctly ad-
justed. Open the key and touch one
electrode to the surface of a cotyledon
of any convenient plant, and the other
to the lower part of the stem. Note
deflection or movement of the electro-

FIG.   44.   Non-polarizable
electrodes.   After Verworn.

meter.   Cut shoots of woody plants and set lower end in a dish
of water.   Place one electrode in water and the other on leaf.
Test the difference between the upper and lower sides of fleshy
leaves.   Test the difference between the base of the midrib of a
large leaf and the middle of the blade to one side of the midrib.

158. Differences in Potential due to Metabolism. Secure a
glass tube 20 cm. long and 4 cm. in diameter. Fuse two glass
tubes 2 cm. in diameter to the sides about 2.5 cm. apart as in
Fig. 5.   Fit caps of rubber over the lateral tubes and perforate
them to allow the passage of the electrodes, which should be
fitted air-tight.   Fit the ends of the large tube with rubber stop-
pers and glass tubes to serve for the conduction of gases.   Con-
nect one of these with a filter pump, and the other with a large
tube containing glass wool saturated with water.   Disconnect
and put a seedling of pea 15 cm. long in the electric chamber, in
such position that the base and middle portion of the stem may

be touched with the electrodes. Now pass a slow stream of air through the chamber, and a movement of the electrometer may be noticed. Connect a hydrogen generator back of the moist chamber and pass a stream of well washed hydrogen (in permanganate of potassium) through the moist chamber and into the electrical chamber. As the hydrogen displaces the oxygen the difference in potential seen at first will disappear, leading to the inference that it was caused by oxidation, though it is by no means to be considered as absolute proof.[1]
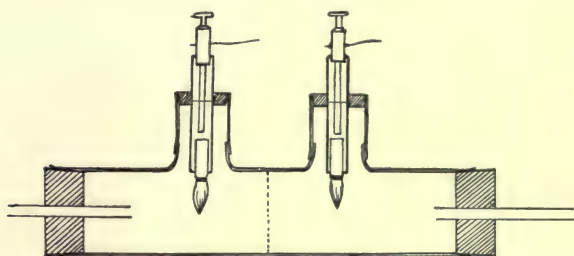
FIG. 45. Electrical chamber for testing relation of oxygen to differences in electric potential. After Haacke.

159. Differences in Potential Between Illuminated and Non-illuminated Portions of a Stem. Set up a preparation as in the previous experiment and note the difference in potential between the regions in contact with the electrodes. Cover the entire tube with cloth, or some device for effectively excluding light, and move to a position to receive the direct rays of the sun. Uncover one end of the tube and allow the light to fall upon the region in contact with one electrode. Cover and a few minutes later repeat allowing the rays to strike the region near the other electrode. A current will be found to set in from the illuminated to the darkened area.[2]

[1] Haacke, O. Ueber die Ursachen elektrischer Ströme in Pflanzen. Flora, 75 : 455. 1892.

[2] Waller, A. D. The electrical effects of light upon green leaves. Abs. Science, 12 : 377. 1900.

Klein, B. Zur Frage ueber die elektrischen Ströme in Pflanzen. Ber. Deut. Bot. Ges. 16 : 335. 1898.

160. Effect of Electric Current on Streaming Movement of Protoplasm.   Secure a leaf of *Philotria* or a stamen hair of *Tradescantia* exhibiting movement, and mount it on a slide with binding post and clips (See Fig. 46).   Place the material so that a minimum amount of water will cover it and the current will traverse the section lengthwise.   Connect a single ammonia cell of the Lelanche or Sampson type with the lower binding posts of

FIG. 46.   Slide fitted with binding posts and clips for sending currents through an object mounted under a cover-glass.   After Arthur.

a DuBois Raymond inductorium with a key in the circuit. Connect the upper binding posts with the fittings on the slide. With a moving strand under observation close the key.   This will cause a primary alternating current to pass through the material.   Note results.

Using the same or a new mount connect the slide with the binding posts of the secondary coil, which should be moved to a
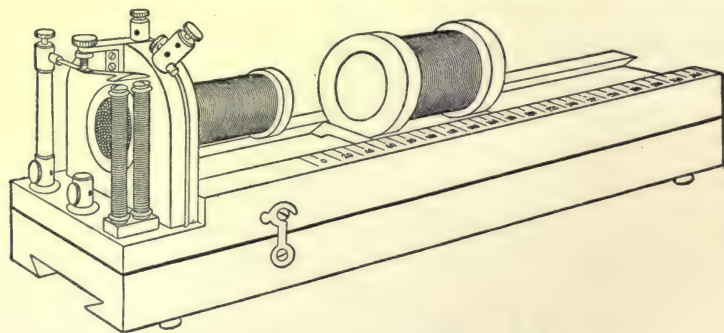
FIG. 46.   DuBois-Raymond inductorium.   After Verworn.

distance of 35 cm. from the primary coil on its sliding base. Close the circuit with the key for five seconds.   Move the secondary coil successively up to 30, 25, 20, 18, 16, 15 and 14 cm., and note results.   After the material has been disorganized by the strongest stimulus describe the condition of the cell.

161. Influence of Induced Current upon Mimosa. Place a number of young plants of *Mimosa* in a warm room with high humidity. Connect the DuBois-Raymond inductorium with the batteries, leaving the key open. Push the secondary coil up over the primary coil and connect the binding posts with the non-polarizable electrodes. Support the electrodes so that one will be in good contact with base of stem and the other with apex. Close circuit for a moment and note result. If no reaction is exhibited, replace electrodes with needles, and after plant has recovered from shock given by thrusting them into the tissues, close circuit again and note result. The outer membrane may prove too highly resistant to secure reaction from the non-polarizable electrodes.

162. Influence of Currents of Electricity Upon Growth : Direct Current. Place 500 or more seeds of mustard, radish, or turnip which possess a germinating capacity of at least 85 to 90 per cent. in water for a period of twelve hours. Take one cc. or more of the seeds and put them into a glass tube of about three-eighth inch diameter (a graduated piece of discarded burette tube will answer best). Solder two copper disks about the size of the inner diameter of the tube to two wires. These will serve as electrodes. Now insert the electrodes into the glass tube bringing them into direct

FIG. 48. *A*, stamen hair of *Tradescantia Virginica* with moving strands of cytoplasm. *B*, same after action of induced current. *a*, *b*, *c*, *d*, irregular masses of cytoplasmic material. After Kühne.

contact with the compacted seeds. Connect the wires to the electrodes with an ammonia, or preferably an Edison-Lalande battery. Place a milliammeter, mercury key rheostat in the cir-

cuit. Stimulate for one minute, with the rheostat adjusted to give a current to two-tenth milliampère. Remove the seeds and carefully select fifty, and place them in a germinating dish, and an equal number of untreated seeds in another dish kept at a suitable temperature, and at intervals of twelve to twenty-four hours note the number of seeds germinated in each lot. At the end of forty-eight or seventy-two hours measure the radicles and hypocotyls, and from the average length obtained determine the percentage of accelerated growth. Determine whether the same results can be obtained by the use of dried seeds[1] (See any handbook of physics).
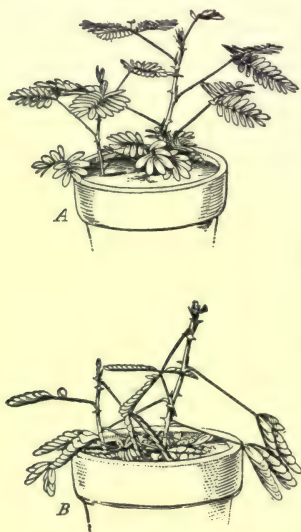
FIG. 49.   A, normal specimen of *Mimosa*.   B, after shock.

163. Effects of Continuous Stimulation. Procure two large glass funnels and support them by glass cylinders. Fill both with earth, and place copper electrodes at the bottom and top of the cone of each funnel. Connect the electrodes of one funnel with a gravity cell battery, leaving the other funnel unconnected. Insert resistance and a milliammeter in the circuit, keeping the current at about two-tenth milliampère. In case the milliammeter is not used determine the E.M.F. and the resistance of the soil, regulating the strength of current with the rheostat. Germinate peas or beans in sawdust, and when the radicles reach a length of one inch, select twenty showing exactly the same development and growth capacity. With a wire make some channels in the soil close to the glass sides of the funnels and insert

[1] Kinney, A. S.   Electro-germination.   Hatch Exp. Sta. Bull. 43.   1897.

the seedling in each, using ten for each funnel. Measure the roots each day and compare the average growth in length of each series. For longer experiments radishes can be grown in boxes of earth provided with copper electrodes, and the weights of the plants determined afterwards. By using copper and zinc electrodes a battery will not be necessary in this experiment, as a current will be generated sufficient to accelerate growth by the action of the soil moisture on the electrodes.

164. Effects of Alternating Secondary Currents. Place one cc. of seeds into the glass tube as in 162 and attach the electrodes to a secondary coil of a DuBois Raymond inductorium as in 161. Attach two cells to the induction coil with a key in circuit and place the secondary coil at 2–4 centimeters from the primary coil. Stimulate the seeds for one minute and note their germination and growth as before. If the same strength of current is used in this experiment as in 163, it will be found that the electrical excitation of an alternating secondary current is greater than that of the primary direct current.

165. Influence of Static Electricity. Construct a small Leyden jar out of a glass cylinder of about 100 cc. capacity by covering the outside with tinfoil. Place a cover on the top, and pass a wire through it. Coil the wire at one end and let it rest on the bottom of the jar, and on the outer end braze a metal bulb. Place some soaked mustard seed in the bottom of the jar and charge from a frictional machine. Remove the seed to a germinator and note the result as before. Compare anodic with the cathodic electrified seeds, and determine whether there is any difference in their germination and rate of growth.

166. Electrotropism. The roots of a number of species are influenced by an electric current in such manner that they tend to direct their apices toward the cathode, within a certain range of strength of the current. The technical unit of strength is a milliampère. The physiological unit used in experimental work is one-millionth as great and is designated by the symbol δ. According to Brunchorst's observations the maximum strength at

which roots of *Phaseolus* seedlings turn toward the cathode is 1.2 at a temperature of 20° C.   If the strength of the current is increased beyond this, mechanical effects due to the disturbance of the conditions of turgidity are produced which may cause a curvature in the opposite direction.   Such curvatures are not to be ascribed to electrotropism.   The maximum currents for *Helianthus* are 1.3 δ, and *Lepidium*, 3.5 δ.

Sporophores of *Phycomyces* exposed to the action of Hertzian waves curved away from the source of the rays much after the manner of apophototropic curvatures. [1]

167. Electrotaxis.   The movement of the entire body of an organism in such manner as to constitute locomotion, in response to currents passing through the medium in which it is found, has
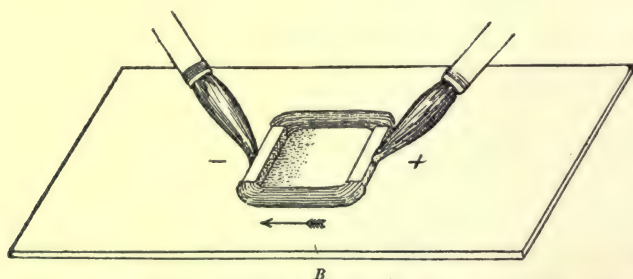
FIG. 50. Cell with walls of rubber and clay for testing electrotaxis of motile organisms.   The electrodes are seen to be applied to the clay walls, and the organisms are aggregated near the kathode.   After Verworn.

been observed only in animals, but the method of investigation is given here with the idea that repeated tests with motile zoöspores and other free moving plants may secure some positive results.   The following method will show the electrotactic movements of paramoecium in a very striking manner.

[1] Hegler, R. Ueber die physiologische Wirkung der Hertz'chen Electricitätszellen auf Pflanzen.   1892.

Loeb, J. Ueber die physiologische Wirkung elektrischen Wellen.   Arch. Ges. Physiol. **69** : 99.   1897.

Brunchorst, J.   Die Funktion der Spitze bei den Richtungsbewegungen der Wurzeln.   Galvanotropismus.   Ber. d. Deut. Bot. Ges. **2** : 204.   1884.

Secure a culture of paramœcium by placing a handful of hay, or decaying leaves, in a large glass jar filled with water from a pond or ditch, standing in a room at ordinary temperatures for a week.

Build a square cell on an ordinary glass microscope slide. To do this place two small strips of rubber or glass parallel with the edges of the slide, and connect the two by similar strips made of moulding clay, or small strips of burnt clay which will become heavily saturated with water. Fill the shallow cell with the culture fluid containing the organisms, place on the stage of a microscope and examine with a lens of wide field, which will give the general form of the paramœcia. Connect batteries of a voltage of 10–15 with the non-polarizable electrodes and then touch the brushes of the electrodes to the pieces of clay. This will send a current directly through the culture fluid. Note the movement of the paramœcia toward the kathode. Break the circuit and note movements. Place a commutator in the circuit and reverse the direction of the current while the electrodes are in contact with the cell.

Repeat with any motile organisms which may be procured. [1]

[1] Davenport, C. B. Electrotaxis. Exper. Morphology. Part I. 1897.

VIII. RELATIONS OF PLANTS TO LIGHT

168. Nature and Derivation of Light. The term light may be applied to all waves of radiant energy included in the spectrum between the infra-red rays with a length of .760 μ, and the supra violet with a length of .397 μ. The light of chief importance to vegetation comes from the sun with a fairly constant steadiness. The movements of the earth however, are such that the intensity of the rays varies through a wide range. The earth is nearer the sun in the summer of the southern hemisphere, and hence plants of that region are exposed to a greater intensity than those of the northern hemisphere during the vegetative season. The inclination of the axis of the earth changes the angle at which the rays strike the surface, thus producing variations in light and temperature, constituting the principal factors in the different seasons. Furthermore the daily rotation of the earth produces a constant change in the angle at which the rays strike the surface of the earth and the plants growing upon it, with the result that the exposure to light varies from darkness to the full intensity of the rays, and back to zero in the course of 24 hours, except, of course, in extremely high latitudes where peculiar conditions prevail. [1]

Local variations in the intensity of light are induced by topographical and meteorological conditions. Light from artificial sources, such as that emitted from flames, phosphorescent substances, the electric arc and incandescent filament, exhibits divergences from the sunlight in the relative intensity of the various portions of the spectrum, a fact that must be taken into account in all experimentation.

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[1] Wiesner, J. Untersuchungen ueber den Lichtgenuss der Pflanzen im Arktischen Gebiete. Sitzungsber. d. k. Akad. d. Wiss. Wien. 109 : May, 1900.

169. Trophic Relations of Light. Light bears a very complex relation to the vegetal organism. It differs from all other trophic factors in the fact that it is not absolutely necessary to the activity and existence of living matter even for extended periods, although it is ultimately of the utmost importance to the plant.

Light exerts a direct chemical effect upon the substances of which protoplasm is composed : it furnishes energy which is absorbed by chloroplasts and is connected with the synthesis of carbon compounds. It stimulates the formation of chlorophyll, although not necessary to the process, and its chemical action disintegrates this substance. The absence of light constitutes a specific stimulus that calls out the various phenomena of etiolation as a reaction, and lastly the rays of light act as a directive or orienting stimulus to which the plant responds by placing its axes at various angles.

170. Tonicity to Light. Not all of the rays of the spectrum are concerned in the various influences exerted by light upon living matter, but only rays of certain wave-lengths are active in each. It is not possible therefore to fix upon a minimum, optimum and maximum of intensity of light which is common to all of the relations between the plant and light. In fact these points may not be distinguished in some of the forms of action enumerated.

171. Direct Chemical Influence of Light upon Protoplasm. Sunlight has been found to exert analytic, synthetic, isomerismic, polymerismic, and catalytic effects upon the chemical substances occurring in protoplasm. How far these changes may be induced when the substances are actually a part of living matter can not be stated definitely. In its synthetic effect light may cause the addition of oxygen to certain organic substances, to which action the fatal influence of light upon certain organisms is supposed to be due. Substances indifferent in darkness unite when their molecules are acted upon by the vibrations of radiant energy. On the other hand many compounds are split into two or more constituents under the same conditions. Hydrogen may be replaced by chlorine, or bromine, in carbohydrates, acids, aldehydes,

ketones, and sulphides.   Actual disintegrations may be induced
among which may be named the breaking down of chlorophyl.

The waves of shortest length and greatest frequency are gen-
erally supposed to be most active in producing these effects,
although it has been proven that rays from the entire range of
the spectrum participate in the disintegration of chlorophyl.

172. Critical Points in the Chemical Action of Light.   No
minimum intensity of light is to be found for non-chlorophylla-
ceous forms since they may exist in total darkness during the entire
period of development of several generations of individuals, or
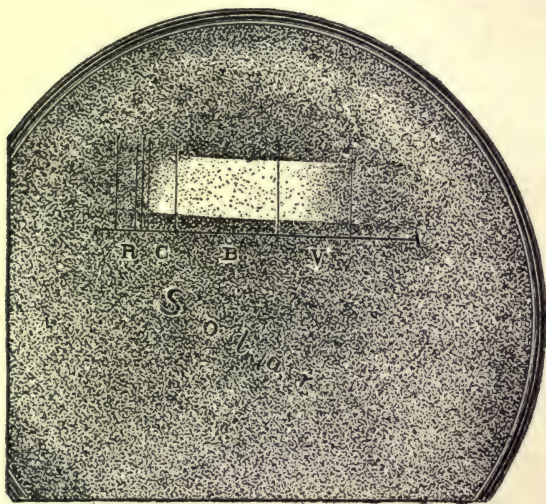
FIG. 51.   Plate of anthrax spores exposed for five hours to the solar spectrum in
August, then incubated for 48 hours.   The horizontal line shows the length of the
spectrum, the vertical lines the limit of the principal regions of the spectrum.   The
letters *R*, *G*, *V* and *B* denote the regions of principal colors, of which they are the
initials.   The clear area is where fewest spores of bacteria have developed after ex-
posure to light.   After Ward.

perhaps forever.   An optimum may be distinguished only for
certain special forms of this class which make use of radiant en-
ergy in the synthesis of foods of which *Bacterium photometricum*
is an example, since none of the direct chemical effects of light

could be of advantage to living matter. The maximum would be reached when the oxidizing effect of light exceeds the capacity of the organism to repair the damage thus caused, or compensate the material broken down. No actual maximal intensities have been determined so far as this phase of the action of light is concerned, although it is known that the blue-violet rays are operative in producing such effects.[1]

173. So-called Rigor of Darkness. A number of lower forms are known to become rigid and inactive when placed in darkness, but actual observation of this phenomenon is mostly confined to such forms as *Oscillaria*, and *Bacterium photometricum*. The manifestations generally classed under the effects of *darkness-rigor* in higher plants include a number of separate reactions. Thus, for example, when a mature green plant is placed in a dark chamber the periodic movements of the leaves soon cease, and the tissues assume a pathological condition and die. Similar behavior is manifested by the same plants in an atmosphere free from carbon dioxide in light, and hence may not be ascribed directly to darkness-rigor. The death of leaves under both circumstances is due primarily to the destruction of chlorophyl, causing a pathological condition of the mesophyl cells of the leaves. *Mimosa* has been cited so much in this connection, that it is proper to say that when a branch of this plant is allowed to develop in darkness not only do the leaves assume a fairly normal stature but they also exhibit periodic motility and irritability to shock and other stimuli.[2]

174. Etiolation. The development of plants in darkness is characterized by alterations in form and structure, as well as in

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[1] Ward, H. M. The action of light upon bacteria. Proc. Roy. Soc. **54** : 472. 1894.

[2] Jost, L. Ueber die periodischen Bewegungen der Blätter von *Mimosa pudica* im dunkeln Räume. Bot. Zeitung. **55**: 17. 1897.

Jost, L. Ueber die Abhängigkeit des Laubblattes von seiner Assimilationsthätigkeit. Jahrb. Wiss. Bot. **27** : 403. 1895.

MacDougal. Relation of the growth of foliage leaves and the chlorophyl function. Jour. Linn. Soc. **31** : 526. 1896.

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variations of irritable properties in the greater majority of species which form chlorophyl screens for the absorption of energy from light. These deviations consist in the suppression or enlargement of leaves, the attenuation of stems, the lack of differentiation of embryonic tissues, the suppression of branching, and various changes in the position and development of the secondary reproductive organs. Fertilization and development of seeds and fruits may or may not occur, according to the amount of development ordinarily attained by the flower before emerging from the bud. The absence of the directive stimulation of light permits organs to assume positions in response to geotropism alone, or to hyponasty or epinasty. Some forms of irritability, such as lateral geotropism and phototropism may be lost, and the entire system of correlations by which a plant determines the relative positions of its various organs may be altered.[1] These reactions are in the main adaptive efforts on the part of the plant for the purpose of raising the chlorophyl-bearing organs past an obstruction intercepting light. The growth of a plant devoid of reserve foodmaterial in darkness also entails starvation. Simple etiolation phenomena should therefore be studied in branches of a shoot extended into a dark chamber, or in specimens placed in darkness having a supply of stored food in the seed-leaves or tubers.

175. Etiolated Seedlings. Place a number of germinating beans, hickory nuts, acorns, or dates in a dark chamber under ordinary conditions of culture, and allow them to reach the full limit of growth, which will need several days, or even a few months in the case of the larger seedlings. The dark room should be provided with double doors tightly fitted to exclude light after the manner of a photographic dark room. If this can not be procured use an ordinary room with all cracks and windows closed and darkened and enter it only at night. Small portable dark chambers of suitable size may be made of wood or sheet zinc set on a table covered with sand. Suitable ventilation must be provided in

[1] Vöchting, H.   Ueber den Einfluss des Lichtes auf die Gestaltung, und die Anlage der Blüthen.   Jahrb. Wiss. Bot. **25** . **149**. 1893.

all instances. The etiolated specimens must not be exposed
to intense light even for a few minutes and the examination of
them should be made by the use of an ordinary candle. The
temperature should be kept at 15–20° C., and not so much water
will be needed as in plants grown in daylight, owing to the les-

FIG. 52. Cross section of portion of normal stem of young *Quercus*. *A*, proto-
xylem. *B*, median part of wood-ring in which the medullary rays may be seen. *C*,
cambium. *D*, bast. *E*, inner periderm.

sened transpiration. Control specimens should be grown under
similar conditions but in full exposure to direct sunlight.

Compare the etiolated and normal stems with regard to the
length and thickness of the stems, length and number of inter-
nodes, number and size of the leaves, and structure of all the

organs of the shoot.   Make a plan showing the relative position
of the shoots and branches.

176. Etiolation of Plants with, and without Aerial Stems.   Se-
cure normal healthy specimens of rhizomes of *Viola obliqua*,
which sends only leaves and flowers above the surface of the soil.
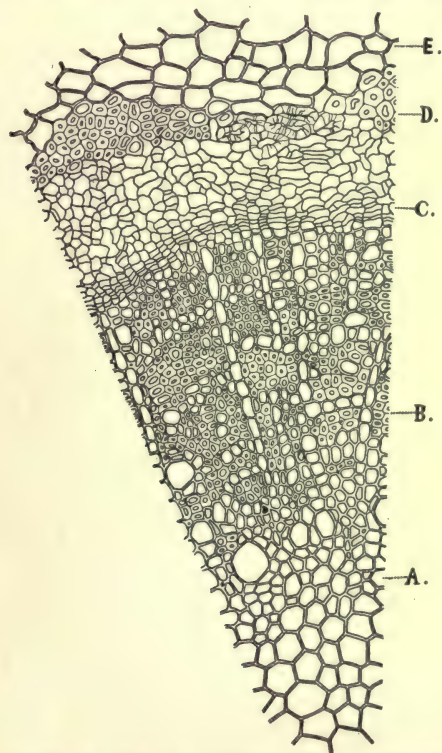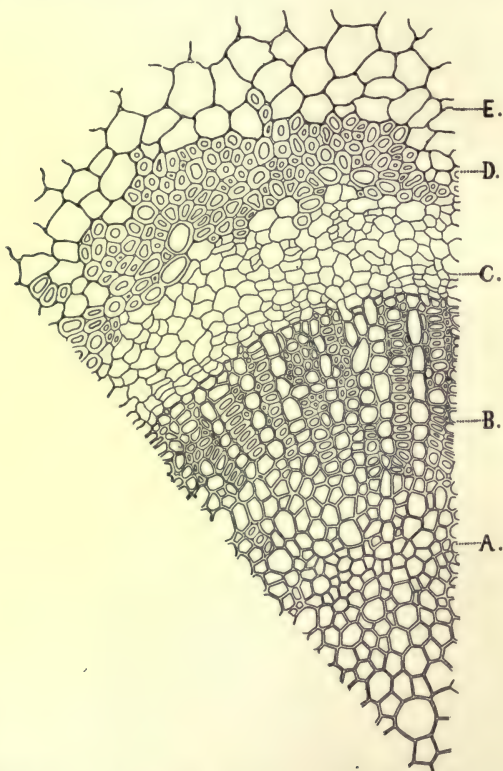

FIG. 53. Cross section of portion of etiolated stem of young *Quercus*. *A*, pro-
toxylem. *B*, median portion of thin wood-ring, in which the medullary rays are
larger than in the normal. *C*, cambiform tissue. *D*, bast, showing greater develop-
ment than in the normal. *E*, inner periderm.

and also a similar number of *Viola rostrata* which forms a leafy
shoot.   Allow these plants to remain in a cold house or out-of-
doors until December 1st, then bring gradually into a forcing

temperature as above. Note the development of both plants in light and in darkness.

177. Etiolation of Leaves with Parallel Veins. Secure a number of bulbs of *Narcissus*, or some similar plant, and force in dark room or greenhouse in January or February. Compare the size, form and structure of the normal and etiolated specimens. Compare also the flowers in the two demonstrations. The tender organs of the etiolated specimens should be supported in order not to suffer damage by bending from their own weight.

178. Etiolation of Sessile Leaves. Force the growth of some beets in dark room and compare with normal individuals.

179. Etiolation of Climbing and Trailing Plants. Grow a number of specimens of *Menispermum*, *Apios*, *Falcata*, or any of the tuberous rooted Convolvulaceae in a dark room. Compare the

FIG. 54. *Viola rostrata* (normal).
FIG. 55. *Viola rostrata* (etiolated).
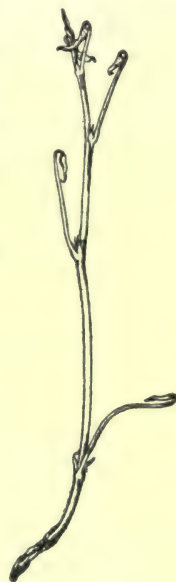
position, form and structure as above. Note also the reactions of the shoot to gravity. Do the nutating movements persist?

180. Formation and Maintenance of Chlorophyl. Chloroplasts are functionally active only when exposed to light, and generally do not construct chlorophyl until stimulated to do so by light, although many forms are capable of building and maintaining this substance in total darkness. It is evident, therefore, that no absolute minimum of intensity for this process is to be found, and no statement may be made as to the optimal stimulating effect. The increase in the intensity of light may reach a point where

chlorophyl is broken down faster than it can be built up, constituting a maximum. This maximum must be regarded as a point at which the constructive efforts of protoplasm under the stimulation of light are overbalanced by the disintegrating effects which are exhibited by rays from all parts of the spectrum. The induced temperatures doubtless play a part in the process. The disintegration of chlorophyl in darkness is probably a reaction on the part of the plant to remove a substance which has become useless and which is maintained at great cost. Some forms, such as the Cactaceae, conifers and ferns, retain the chlorophyl unchanged, however.[1]

181. Formation of Chlorophyl in Darkness. Secure some healthy specimens of *Botrychium, Osmunda,* or *Aspidium* or any convenient fern and remove them from the soil, if out of doors about December 1st, and set in flower pots of suitable size. Bring into forcing room and dark room gradually. Note general form of etiolated specimens and also the presence of chlorophyl.

FIG. 56. *Viola obliqua* (etiolated).
FIG. 57. *Viola obliqua* (normal).

Examine the chloroplasts and compare with those of the normal individuals. Germinate seeds of *Pinus, Thuja* or other coniferous trees in soil in dark chamber.

182. Growth of Green Plants in Darkness. Place a number of nearly mature plants of *Narcissus,* or *Arisaema,* in the dark chamber and note the behavior of the leaves in regard to growth, and persistence of the chlorophyl.

[1] See Pfeffer, W. Plant Physiology, **1** : 233. **1900.**

183. Formation of Chlorophyl in a Blanched Specimen. Place a small artificial light such as might be produced from an oil flame or a few candles, or a single incandescent bulb, in a dark chamber containing etiolated plants and note the formation of chlorophyl. Bring an etiolated plant from the dark room and note the time in which a greenish tinge will be taken on by the leaves.

184. Microchemical Test for the Presence of Chlorophyl. Secure thin leaves of mosses, or of some aquatic, or mount a thin section of some leaf on a glass slip. Place a drop of saturated solution of potassium hydrate in water, on the section and examine with a microscope. Chloroplasts will take on a yellowish brown color immediately, which will change to a green tint in the course of half an hour. This change may be hastened by the addition of a drop of glycerine or alcohol run in under the cover-glass.[1]

185. Absorption of Light by Tissues of Plants. A simple diaphanascope may be made from two shells of cartridges used in shot guns. One should be 10 gauge and the other 12 gauge and should be uncapped.

Cut circular pieces of leaves of proper size and place over the end of the smaller shell then slip the larger shell over it and hold the instrument toward the sun. Note the amount of light transmitted through a single leaf. How many leaves are necessary to exclude the light completely. Test tissues from other parts of the body of the plant. Test the permeability of red leaves.[2]

186. Purposes and Uses of Chlorophyl. Chlorophyl is an extremely complex and unstable substance or group of compounds

[1] Molisch, H. Eine neue mikrochemische Reaction auf Chlorophyll. Ber. Deut. Bot. Ges. **14** : 16. 1896.

For the general chemistry of chlorophyl see Marchlewski, Chemie des Chlorophylls. 1895. And Jour. f. Prakt. Chem. **62** : 247. 1900.

Etard. Pluralité des chlorophylls. Compt. Rend. **120** : 328. 1895.

Gautier. Sur la pluralité des chlorophylls. Compt. Rend. **120** : 355. 1895.

[2] Linsbauer, L. Untersuchungen ueber die Durchleuchtung von Laubblättern. Beihefte, Bot. Contralb. **10** : 53. 1901.

which appears to have been developed for the purpose of acting as a light-absorbing screen. The absorption by chlorophyl and its derivatives, or accompanying substances, does not affect the whole spectrum, and is greatest in seven different regions. The energy derived from the radiations absorbed is used in splitting apart the simple compounds taken up by the plant, and allowing their unsatisfied chemical affinities to form new and more complicated compounds of great potential energy, constituting the process of photosynthesis. The construction and arrangement of the organs of the plant to obtain the proper exposure of chlorophyl has been the most important factor in the development of the shoot.

187. Critical Points in the Photosynthetic Relations of Light to Plants. A minimum intensity of light below which energy ceases to be absorbed and used is not easily distinguishable. The diffuse rays of moonlight which have only the intensity of one six-hundred-thousandth of daylight are doubtless sufficient to furnish enough energy for some photosynthetic action, but it may not be estimated since the amount of carbon dioxide used and oxygen given off would be far overbalanced by the respiratory interchange. There is doubtless a minimum more or less adjustable below which every species may not continue existence indefinitely, but it does not lend itself to physical measurements.

The optimum intensity for photosynthetic action is about that of direct sunlight in the temperate zones. Generally a marked increase over the optimal intensity must be made to exert a lessening effect upon photosynthesis. Reinke found that the intensity must be increased sixty times before a decrease was shown by *Philotria*. The maximum is equally intermediate with the minimum, although it [1] is well known that any given species cannot survive uninjured for any extended period in an intensity above its accustomed standard. All of these critical points are greatly influenced by other trophic conditions such as moisture and temperature. The amount of light actually impinging upon

[1] Pfeffer, W.   Plant Physiology, 1 : 340. 1900.

the chloroplasts in any plant is always decreased by the opacity of the outer membranes.   Beyond this however, it is found that the amount of light absorbed does not correspond exactly with the amount of photosynthesis.

188. Fluorescence of Chlorophyl Solutions.   Place 100 grams of freshly chopped young leaves of any convenient species, the sap of which gives a neutral or alkaline test with litmus paper, in an evaporating dish and cover with distilled water.   Boil for
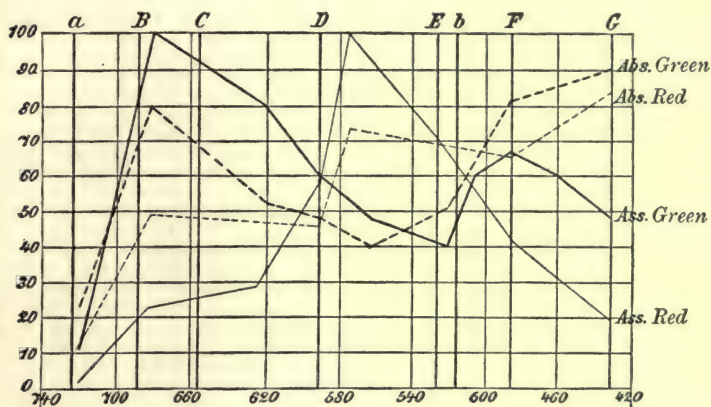

FIG. 58.   *Abs. green*, curve showing amount of energy absorbed from different portions of the spectrum by green chloroplasts.   *Ass. green*, amount of photosynthesis in same portions.   *Abs. red*, curve of absorption by red algae.   *Ass. red*, amount of photosynthesis in corresponding portions of the spectrum.   Engelmann, after Pfeffer.

half an hour.   Pour off the water and wash repeatedly in distilled water.   Squeeze out the last of the water and place the material in a closed flask and cover with 500 cc. alcohol (95 percent.).   Set in a dark place and shake occasionally.   A serviceable solution of chlorophyl will be obtained in a few hours.   Decant some of the solution into a narrow test-tube, and hold between the eye and a strong light at various angles until a blood-red fluorescence can be seen at the edge of the solution.   The effect may be heightened if the test is made in a dark room and a small beam of daylight admitted.   This fluorescence is due to

the capacity of chlorophyl for absorbing rays of one wave-length and emitting others of greater length, or of converting rays from the upper part of the spectrum to the lower red.

189. Absorption Spectrum of Chlorophyl. Adjust an Abbe, or any convenient spectroscopic eyepiece to a microscope fitted with a low power objective. The instrument should stand on a table in a strong diffuse light and the mirror of the micro-
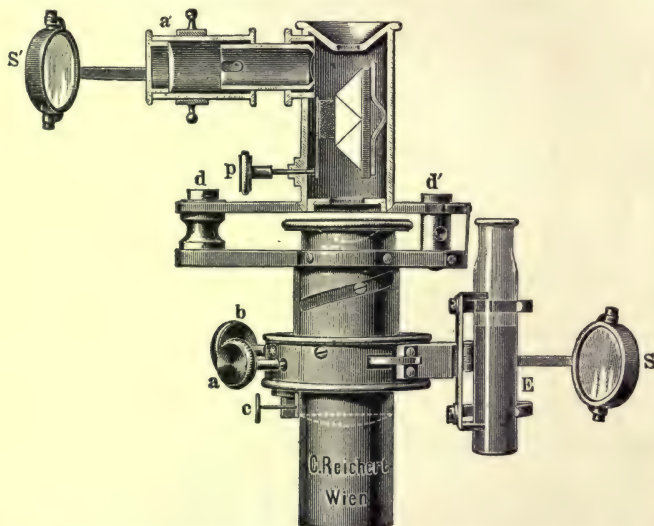

FIG. 59. Abbe micro-spectroscope. *a*, *b*, screws for regulating the slit through which light passes. *c*, screw for clamping apparatus to tube of microscope. *d′*, spring which, loosened, allows the ocular portion to swing around on the pivot, *d*. *E*, phial containing solution to be tested. *S*, mirror for reflecting light upon solution. *S′*, mirror for illuminating scale. *p*, screw for manipulating Amici prism, and the extended drum into which the screws *a*, and *b*, project contains the comparison prism which receives light from the objective of the microscope, and throws a solar spectrum alongside that which has come from the light passing through the solution at *E*.

scope should be arranged to give a plain solar spectrum. Light from the sun should be thrown on the lateral mirror of the spectroscope by means of a heliostat, or a strong artificial light from a welsbach or argand burner should be provided. The spectrum of the second should be of the same intensity

as the first, and both may be regulated by the adjustment screws of the spectroscope.   The spectroscope should be provided
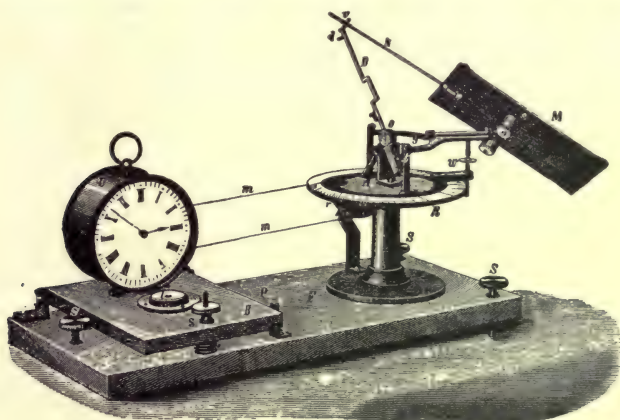

FIG. 60.   Heliostat, for reflecting a beam of sunlight from the mirror *M* constantly upon one spot.

with small bottles of several sizes for holding solutions of chlorophyl.   Fill the thinnest of these with the solution in alcohol and adjust to the instrument.   Compare the spectrum of the light

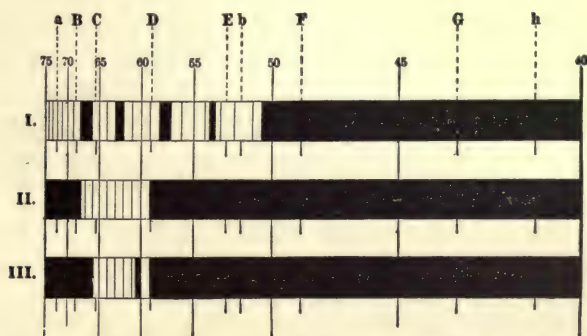FIG. 61.   *I*, spectrum of chlorophyl.   The three indistinct bands beyond *E* are shown as one, and are not usually distinguishable with the micro-spectroscope.   *II*, spectrum of amaranth-red in which nearly all of the rays except those between *B* and *D* have been absorbed.   *III*, spectrum of autumnal coloring matter of *Ampelopsis*. Nearly all of the light except a portion between *C* and *D* has been absorbed.

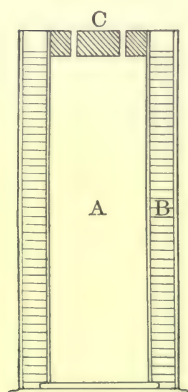that has passed through the solution with the solar spectrum. Note the presence of several bars or bands (black or dark) cross-ing the spectrum. Adjust the width of the slit and the dispersion until these are seen most distinctly. If an Abbe spectroscope is used set the scale so that " 75 " marks the lower edge of the red color. Draw a similar scale on a sheet of paper and plot the absorption bands seven in number. Probably not more than three or four may be seen simultaneously. Repeat with other bottles containing layers of fluid of different thicknesses. Also vary the concentration of the solutions, and all may be finally made out. Very satisfactory results may also be obtained from the use of a direct spectroscope of the pattern supplied to physical laboratories.

FIG. 62. Appa-ratus for exposing plants to separate por-tions of spectrum. *A*, glass cylinder fitted with perforated stop-per, and weighted with lead, placed in a larger cylinder. Col-ored fluid is poured in the outer cylinder until it rises to the level of the stopper and a cover of tinted glass is laid over the whole (see color fil-ters).

190. Action of Light on Chlorophyl Solutions. Extract the chlorophyl from boiled leaves by means of sulphuric ether instead of alcohol. Secure an alcoholic solution of equal depth of color. Divide both solutions into two lots and thus fill four test-tubes. Place one each of the alcoholic and ether solutions in a dark chamber for a day, and the others in a strong light. Compare the action of the light in the two solutions and note the difference in color of the solutions kept in the dark and in the light.

Fill four test-tubes with alcoholic solution and cork two of them tightly. Expose and open closed tube in light and darkness. If double-walled bell-jars or the apparatus in fig. 62 is used, a test may be made of the influence of red and blue light in producing this deterioration. The temperatures set up, however, are such that the experiment is of but little final value (See color filters).

191. Red and other Coloring Matters in Leaves. Cut sections
of red leaves of *Achyranthes*, or *Coleus* and note the character
and location of red coloring matter in the leaves. The presence
of these substances implies that only a portion of the spectrum
is transmitted to the chloroplasts beneath.

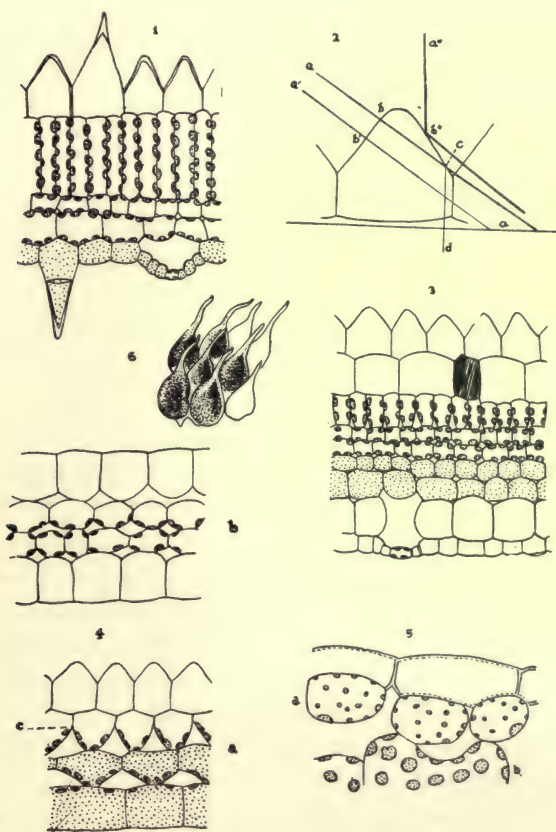Fig. 63.   1. Transverse section of a velvety leaf of *Eranthemum Cooperi*. The
epidermal cells of the upper side are furnished with elongated papillose extensions
for entrapping sunlight.   The extremities of some cells are converted into hairs.   The
epidermis of the lower side contains anthocyan.

   2. Diagram showing the manner in which light enters the epidermal cells of velvety
surfaces.

3. Transverse section of a velvety leaf of *Piper porphyraceum*. A layer of aqueous tissue lies next the epidermis of the upper and lower sides. The anthocyan is in the lower half of the leaf.

4. Mottled leaf of *Begonia falcata*. *a*, transverse section of a brownish green velvety shining portion of lamina. The epidermal cells of the upper side are furnished with papillose extensions. The epidermal and subepidermal layers are joined without intercellular spaces. The epidermis of the lower side and the spongy parenchyma contain anthocyan. *b*, transverse section through a silvery portion. The outer walls of the epidermis are plane. Large air spaces are present between the epidermis and the cells containing chlorophyll.

5. Transverse section of a bright spot on the leaf of *Ranunculus ficarioides*. The subepidermal cells *a*, contain a few small chloroplasts, and are separated from the layer beneath by large air-spaces.

6. Papillose epidermal cells of *Begonia imperialis*, var. *smaragdina*, seen from above by refracted light. After Stahl.

Boil a number of colored leaves, *Amarantus*, in distilled water until a concentrated deeply colored solution is obtained. Test with the spectroscope and note absorption bands, which are not easily made out. Or place a number of red leaves of *Coleus* in a jar with ether vapor for 20 minutes, then chop fine and extract with distilled water.[1]

192. Relation of Anthocyan to Light. Secure two leaves of Canna or cabbage alike in all particulars except that one contains a large amount of red coloring matter (anthocyan) in addition to the chlorophyl, which is present in about the same quantity as in green leaves. Wrap each leaf around the bulb of a long thermometer and expose to sunlight. It is important that the same number of thicknesses of similar portions of the leaves should be interposed between the light and the bulbs. Read the thermometers in half an hour and note the influence of the red color.

193. Arrangements for Concentrating Rays on Chlorophyl. Cut a cross section of a leaf of *Coleus, Cissus, Begonia* or any leaf showing a velvety upper surface and examine the contour of the epidermal cells. The outer walls are seen to be convex and capable of converging all of the rays which strike the surface of the leaf at any angle upon the layer beneath containing chlorophyl.

[1] Müller, N. J. C.   Spectralanalyse der Blüthenfarben.   Jahrb. Wiss. Bot. **20** : 78. 1889.

194. Stimulating Influence of Light.   The absorption and use of the energy of light depends upon the angle with which the rays strike the surfaces, and the intensity of the impinging rays. A few species of green plants have become adapted to living in faint diffuse light, but the greater majority find their optimum in direct sunlight.   In order to be able to attain the most advantageous positions it has been necessary for the plant to acquire irrito-motility to light, and to be able to place its body at proper angles to the impinging rays.   Species which have a habit of clinging closely to a horizontal or vertical substratum or support, tend to move their bodies away from the source of light as is also the case with typical roots constituting *aphototropism*, while shoots generally tend to move toward the center of the radiations because of their *prophototropism*.   The organization of the shoot
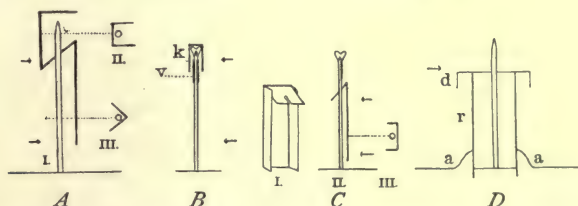

FIG. 64. Diagrams of shields for allowing phototropic stimuli to fall on restricted regions of a seedling. *A*, paper shield adjusted to seedling of *Avena* exposing the basal portion to action of light the direction of which is indicated by the arrows. Cross section of tip at *II*, and basal portion at *III*. *B*, a band of tinfoil is wound tightly around a hypocotyl of *Brassica* at *v*, and a free part is twisted into a cap which fits tightly over the cotyledons at *k*. *C*, black paper shield for covering basal portions of hypocotyl of dicotyledonous plants. *D*, cylinder of tinfoil *r*, to surround basal portion of seedling of *Avena* with cap *d*, through which the apical portion projects. *a, a,* level of the soil.   After Rothert.

is such that this generally carries the leaves into a zone of stronger illumination.   Leaves exhibit still a different form of re- action by which they place their surfaces at right angles to the direction of the rays in response to their *diaphototropism*.   This form of irritability is also exhibited by many zygomorphic flowers. Phototropism is also exhibited by some chlorophylless forms.   The reactions in such organisms are generally of advantage in the dis-

tribution of spores.   It has already been pointed out that light
may also determine the direction in which a reaction movement
may be made in response to gravity (See diageotropism of
flowers of *Narcissus*).

195. Perceptive Zones in Phototropism.   Not all of the parts
of the organs of the shoot are equally sensitive to the stimula-
tion of light.   In general it is found that the apical part of a
stem is most sensitive, but the power of receiving the stimulus
is generally shared by the older parts, sometimes with an equal
degree of delicacy, though in other instances the power of per-
ception is less as the distance from the tip increases.   The seed-
lings of the Paniceae alone show a restriction of phototropic sen-
sibility to the cotyledons.

196. Localization of the Sensory Zone.   Germinate a number
of seeds of *Avena sativa* (oats) in a shallow pan in a dark cham-
ber.   When the plumule has reached a length of three cm. cover
the tips with a cap made of tinfoil or black paper.   The caps may
be made by rolling squares of tinfoil around the hypocotyl like a
cigarette paper, and then closing one end by pinching and bend-
ing (Fig. 64, *B*).   The caps should fit tightly over the tip,
and should cover six or seven mm. of the terminal portion.
Provide half a dozen plants with such coverings and set with an
equal number of untreated specimens in a phototropic chamber.
The phototropic chamber consists of a tightly made box lined
with black cloth, with a length of 60 cm., a width of 30 cm.
and a height of 30 cm.   One end should be hinged and should
be movable like a door closing against the padded edges of the
box in such manner as to exclude all light.   A circular opening
should be made in the door about eight cm. above the bottom
of the box, and a shallow tin or wooden box of suitable size
fastened over this opening in such manner that it will hold a
flask with parallel walls to contain colored fluids.   The tin is
fastened to the door by its edges, which are padded to prevent
the passage of light, and an opening is made in the bottom of the
tin box to correspond to that in the door.   A hole should be

bored in two sides and the top and rubber tubes inserted and fastened in a curved position in such manner that ventilation is secured without the admission of light. After the chamber has been made, take it into a dark room, close the opening in the door by means of a stopper, and put a piece of photographic paper inside. Set in direct light for an hour, then examine paper in dark room and note if it has been acted upon by light.

When the seedlings have been placed in the box it should be set in an exposed position with a mirror or heliostat arranged to throw horizontal rays into the opening. After five hours open and note positions of cotyledons. Those covered by the tinfoil will have made but little curvature, while the normal specimens will show a noticeable curvature toward the light.
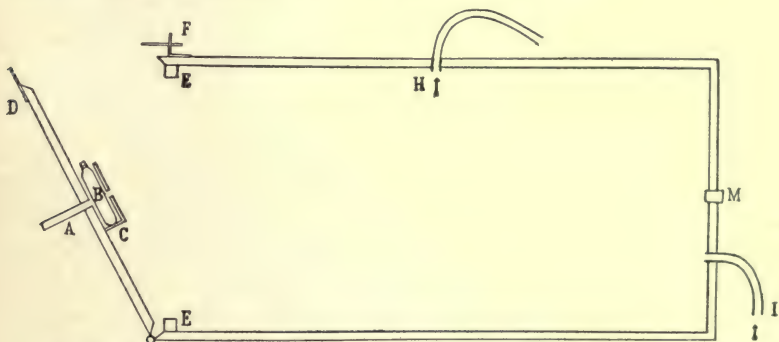

FIG. 65. Phototropic chamber. *A*, tube admitting light which passes through the flask *B*, containing colored liquid. *E, E*, cleats with packing on outer faces against which the door closes tightly, and is held by the bolt *F*, which is pushed through a hole at *D* and secured by a nut. *H, I*, ventilating openings. *M*, opening which may be closed with ordinary stopper, or receive a second tube.

Repeat this experiment with seedlings of *Phalaris Canariensis.* Next cover the basal portions of another set of seedlings by cylinders of tinfoil or black paper and compare results. It may be seen that the region of the tip alone is sensitive to light and that when this is covered no reaction occurs. The region of extreme sensitiveness does not include more than about 3 mm. of the tip of the cotyledon.

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197. Transmission of Stimulus-effects.   If the cylinder of tin-foil employed in the latter portion of the above experiment should cover all the seedling except the extreme tip it would be found that a reaction curvature would take place in a portion not directly exposed to the action of light, demonstrating that a transmission of the effects of the stimulus has taken place.   Transmission toward the tip from a basal portion of a shoot has not yet been observed.   It has been found that transmission takes place through the living parenchyma cells of the fundamental tissues.

198. Transmission in Stems.   Strip a plant of *Coleus* of all of its leaves and place it in a dark room for a day.   Cover all of the stem except the apical internode with tinfoil, or pile sphagnum around it and bind with rafia fiber to effect the same purpose.   Place the preparation in a phototropic chamber, or in an open room where it will be illuminated from one side only.   Note the position of the stem a day later.[1]
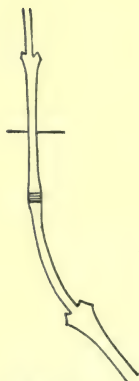
Fig. 66. Stem of *Coleus* curved phototropically after exposure of the terminal portion only. After Rothert.

199. Rays Inducing Phototropic Reactions.   Place six seedlings of any of the species used in the previous experiments in the phototropic chamber and put a flask with parallel walls in the receptacle in the door.   Fill the flask with an ammoniacal solution of copper oxide.   This solution may be prepared by adding an excess of ammonia to a watery solution of copper sulphate, and should be of such concentration that a printed page may be read through the flask containing it.   Set the apparatus where a strong light may be thrown into the chamber passing through the solution in the flask.   Examine six hours later, and note the angle of curvature.

Replace the seedlings with a second lot and refill the flask with a saturated solution of potassium bichromate in water.   Note the

[1] Rothert, W. Ueber Heliotropismus. Cohn's Beitr. z. Biol. d. Pflanze, 7: 1. 1896.

amount of curvature six hours later. The test will have greater value if two chambers are provided and the tests are made simultaneously, with lots of seedlings that have stood in a dark room for 24 hours. Examine the solutions by means of the spectroscope and ascertain what rays are absorbed and what are transmitted by each. This will lead to conclusions as to the part of the spectrum most active in inducing phototropic reactions.

200. Color Filters.[1] Red. .05 g. water free cantharides green crystal violet dissolved in sufficient alcohol. Dilute to 1 L. with distilled water. In a layer of 20 mm. thickness a red and wide blue violet band is given. The latter may be removed by a solution of potassium chromate 10 to 100 water in a layer 20 mm. in thickness.

Yellow. Dissolve 30 g. of crystals of nickel sulphate in 100 cc. of distilled water. Used in a layer 20 mm. thick it absorbs red only. Now pass the light through a solution of potassium chromate 10 g. in 100 cc. of water in a layer 15 mm. thick. This will absorb the blue. Then pass the remaining light through a solution of potassium permanganate .025 g. in 100 cc. of water. Only orange yellow and a trace of red will remain.

Green. Dissolve 60 g. crystals of copper sulphate in 100 cc. of water and use in a layer 20 mm. thick. Only green and blue pass through it. The latter may be taken away by the potassium chromate solution described above. A wide green band with a trace of red remains.

Blue, bright. Dissolve .02 g. of methyl green (Doppel-grün, S. F.) which will give a bright bronze precipitate with chloride of zinc, in 100 cc. of water. Used in a layer 20 mm. thick it allows red and blue to pass. The red may be taken away by a solution of 15 g. copper sulphate (crystals) in 100 cc. water in a layer 20 mm. thick.

Blue, dark. Dissolve .005 g. crystal violet 5 BO in 100 cc. water and use in layer 20 mm. thick. Also 15 g. copper sul-

[1] Methods for obtaining pure colors used by II. Landolt in some work on "rotations dispersion." Ber. Deut. Chem. Ges. **27** : **2872**. **1894**.

phate in 100 cc. water and use in layer 20 mm. thick. The potassium permanganate should be freshly made when used, and the color solutions should be kept in the dark or in opaque glass bottles.

201. Reaction Time. Bring a rapidly growing plant from the dark room in which it has been placed for a day, and set it near a window from which it will receive a strong light. Set a horizontal microscope with its barrel parallel to the window and quickly focus on some part of the tip of the terminal bud. Note the length of time elapsing before movement of shoot toward source of light takes place. Seedlings a few centimeters in height will be most suitable for this test.

202. Critical Points in the Phototropic Relations of Light to Plants. The intensity of light necessary to constitute a stimulus varies enormously in different species according to the degree of sensitiveness which they have acquired and the stage of development. A "normal" candle burning 7.78 grams of paraffine per hour and standing one meter from the sensory zone may be taken as a standard. The intensity of illumination decreases as the square of the distance from the flame. *Lepidium sativum* has been found to respond to .00033 meter candle illumination in a dark room, and the minimum varies in different species to .06 meter candle in *Raphanus sativus* and others. The intensity necessary to secure the fullest reaction constituting the optimum varies from .11 meter candle in *Pisum* and *Phaseolus* epicotyls to .44 meter candle in the epicotyl of *Vicia.* The optimum is generally much higher in etiolated plants, which are also less sensitive to geotropic stimuli. An increase of the intensity of illumination a hundred or even a thousand times is necessary to reach the maximum, or point beyond which the reaction ceases.[1]

Increase of the intensity of illumination above the maximum

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[1] Figdor, W. Versuche ueber die heliotropische Empfindlichkeit der Pflanzen. Sitzungsber. Akad. d. Wiss. Wien. **102** : 45. 1893.

Wiesner, J. Photometrischen Untersuchungen auf Pflanzenphysiologischen Gebiete. Sitzungsber. Akad. d. Wiss. Wien. **102** : 291, 350. 1893.
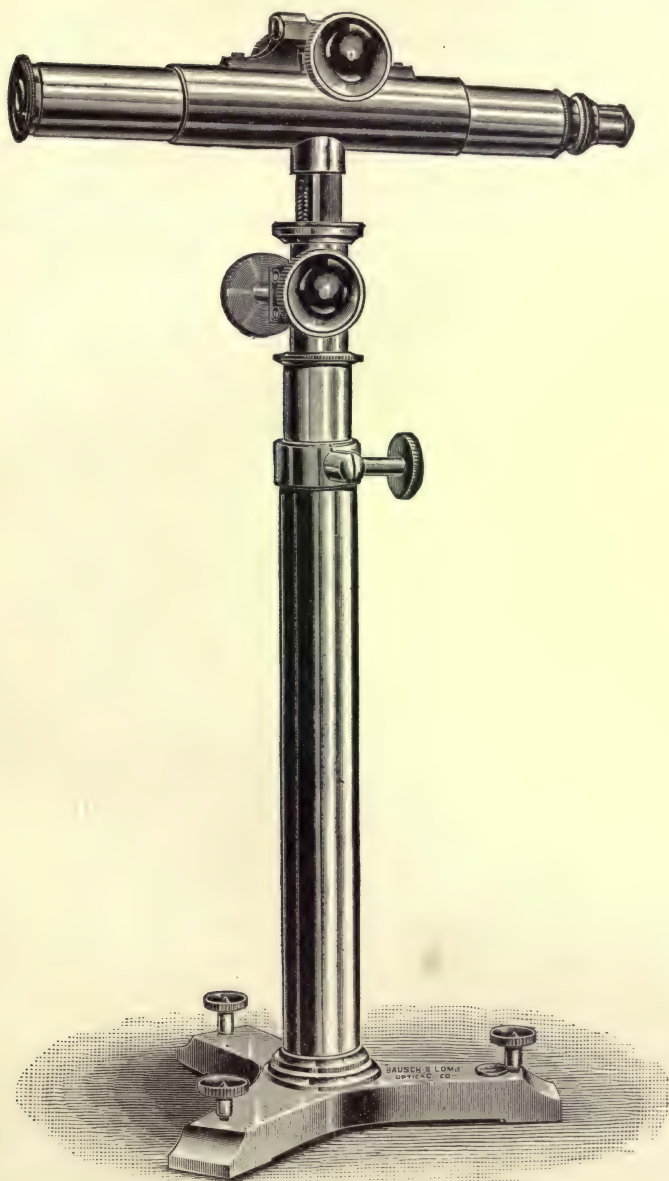
FIG. 67. Horizontal microscope. After Barnes.

may have the effect of changing the character of the response and the resulting curvature may carry the organism away from, instead of toward the source of light. This is true of free moving organisms which have a low photosynthetic optimum. In fixed forms the reaction consists in moving the organs to place their surfaces more nearly parallel with the rays.

The amount of increase in illumination necessary to constitute a stimulus has been found to be very slight in the few organisms in which it has been investigated. Results at hand are fairly conclusive that accretions to constitute a stimulus are in accordance with Weber's law.[1]

203. Intensity of Illumination Necessary to Constitute a Stimulus. Grow seedlings of *Avena*, or *Phalaris*, in a dark room, by the use of germinating pans. When the shoots have reached a height of two cm. place an ordinary paraffine candle at a distance of three meters and allow it to burn for an hour. The rays of light should strike the upper part of the seedling and leave the lower part in the shade. Extinguish the candle and examine the seedlings at the close of a second hour. Repeat the test, moving the candle farther away from the seedlings every time until no reaction is secured. It may be more convenient to use the smaller candles sold for Christmas decorations or a micro-gas-burner. The candles may be standardized as above, or the gas-burner by means of a photometer in the physical laboratory.

204. Negative Reactions to Light above the Maximum. Obtain some mud and water from a ditch containing numerous specimens of *Euglena viridis*, and place in an earthenware, or porcelain dish and set near a south window for a few days. Remove the culture to the middle of the room and take up a few drops of the liquid and place a suitable amount containing numbers of the *Euglena* on a large cover-glass, and invert over a stage moist chamber after the manner of a drop culture. Examine with a low power and

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[1] Massart, J.   Recherches sur les organismes inférieurs.   La loi Weber vérifié pour l'héliotropisme du champignon.   Bull. Belg. Acad., **16** : 590.   1888.

note the distribution of the organism through the culture. Now move the microscope to a distance of three meters from the window and look for changes in distribution. A half hour later move to within 1.5 meters of the window and observe. Next move directly up to window, but not in sunlight. If suitable conditions are offered the maximum may be found, and as the preparation is brought nearer the window the organisms move away from the source of light, thus exhibiting *aphototaxis*, while at lower intensities they were prophototactic. Interesting tests may be made with zoöspores of all kinds.[1]

205. Summation of Stimuli. Grow seedlings of any convenient species in the dark room until they have attained a height of 2–4 cm. and then expose to illumination of a small candle or burner, to ascertain the minimum amount of time necessary to secure a reaction. Now secure a second lot of seedlings, and expose them to an illumination of the same flame for a period of one-fifth of the presentation time determined, then shade the flame for a period equal to one-tenth of the presentation time, repeating the alternation of periods of illumination and darkness a half dozen times in an effort to ascertain what repetition of an amount of light, not sufficient to produce a response, may secure a response by a summation of effects.

The experiment may be made in another form if an electric sparking apparatus is at hand. The seedlings may be subjected to the illumination of a definite number of sparks at regular intervals.

206. Threshold of Stimulation. When a plant is subjected to light from one source striking it on one side, a certain increase over this intensity will be necessary in a light coming from the opposite direction in order to set up a new reaction. Place a candle or microburner at a distance of three meters from a seedling in a dark room until a curvature is produced, then set a sec-

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[1] Holt, E. B., and Lee, F. S. The theory of phototactic response. Amer. Jour. Physiol. **4** : 460–481. 1901.

Oltmanns, F. Ueber positiven und negativen Heliotropismus. Flora, **83** : 1. 1897.

ond on the opposite side at a distance of two meters.   If a reaction to the second is secured, repeat the test moving the second candle farther away until the response disappears.   Note the difference in the distances of the two candles, and calculate the percentage of difference necessary to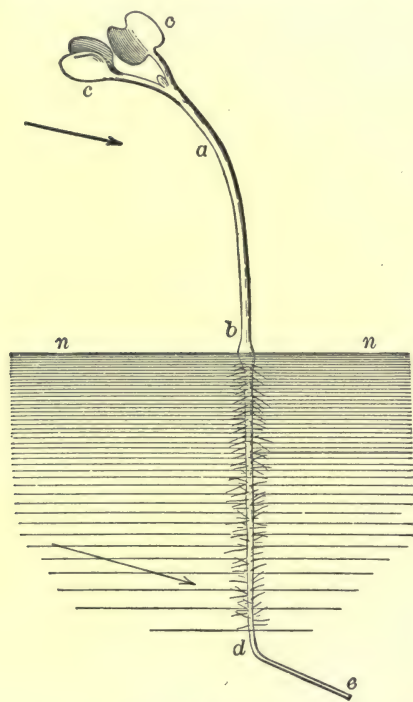 constitute a stimulus.   If no reaction is secured to the second candle at two meters, it must be moved closer. Fresh lots of seedlings should be used in every test, or several hours allowed to elapse between tests if the same are used.

FIG. 68.   Seedling of *Sinapis alba* in dish of water exposed to light from one direction only, indicated by arrows. *c*, *c*, diaphototropic cotyledons, from which the impulse is transmitted to the motor zone of the hypocotyl at *a*. *b*, base of hypocotyl. *n*, *n*, level of water. *d*, motor zone of root, showing aphototropic curvature.   This curvature was produced some time earlier, and the perceptive zone of the root has been carried some distance away by the growth of the tip.   After Sachs.

207. Zone of Curvature, Secure a number of young plants of *Helianthus, Zea, Avena, Lepidium* and others and mark the tips of the stems into intervals of 5 mm. by means of India ink applied with a thread. Grow the specimens in the dark room for a day to relieve the apical regions of all phototropic curvatures.   Measure the distances between the intervals on the stems, and set the plants in a position before a window through which they will receive a strong illumination. Note the region of curvature a few hours later and find what relation it bears to the zone of greatest growth.

208. Aphototropism. Germinate seeds of *Sinapis alba* in saw-dust, or loose soil, and when the roots are about 2 cm. in length take up the seedlings and pass them through holes of proper size in a thin plate of cork, where they are supported by pack-ing of cotton wool. Fill a tumbler with water to within about 3 mm. of the top and set the cork over the mouth with the roots fully immersed in the water, and the entire axis of the plant in a vertical position. Set the preparation in a room at 18° C. near a window where it will receive light from one side only. This may be best accomplished by placing the preparation in the photo-tropic chamber from which the door has been removed and the open end directed toward the light (Fig. 68).

FIG. 69. Shoot of *Helianthus* which has been placed in a horizontal position and illuminated from above. The diageotropic and diaphototropic movements of the leaves have been accomplished by curvatures and torsions of the petioles.

209. Diaphototropism. Probably all dorsiventral leaves tend to place their axes at right angles to the incident rays of light, with the inner (upper) surfaces exposed to the direct action of the rays. If the leaf has an exposure including the whole horizon it will lie in a horizontal position, which might be due also to diageo-tropism. The crowding of leaves under the shadow of other or-gans of the same plant, or of surrounding vegetation however, alters its horizon, in consequence of which it assumes various positions with respect to the vertical, but at right angles to the direction from which its optimum illumination is derived in what

is generally known as the fixed light position. When the body of a plant is moved in such manner as to disarrange the leaves with respect to the light equilibrium, curvatures ensue, to restore the chlorophyl-bearing portions of the organ to their original positions. Here, as in diageotropic reactions, torsions may also accompany the reactions of adjustment to light. The cause of the horizontal position of dorsiventral organs, including stems, thalli and other structures may be determined only by actual analysis. The unequal growth of the two flanks (epinasty, hyponasty) of such organs may also play a part irrespective of external inductions.[1]

210. Paraphototropism of Leaves of Taraxacum. Secure a few young plants of *Taraxacum* in which the rosette includes a number of vigorously growing leaves, which usually lie flat upon the surface of the soil. Transfer them to pots filled to heaping with soil, or enclose the roots in a compact mass of damp sphagnum. Place in a position near a window where the illumination will be strong and from one side only. Note the positions of the leaves a day or two later. Place the plant in an inverted position in a phototropic chamber where it may receive illumination from below at right angles to the dorsal surfaces of the leaves. Note position a day or two later. Close the chamber, shutting out light, and observe position of the leaves after an equal period. Place a plant in a dark room with the root in a horizontal position, and the leaves vertical with respect to their planes. Note position of leaves two days later. Fasten a plant to the clinostat in the last named position and revolve it on its axis. An analysis of the above results will show that the leaves of *Taraxacum* are diaphototropic, apogeotropic, and epinastic.[2]

211. Diaphototropism of Leaves of Arisaema. Place an awakening corm of *Arisaema* in a pot filled with soil and covered with sphagnum and fasten to a clinostat in such manner that the main axis of the plant is horizontal and perpendicular to a win-

[1] Ewart, A. J. Diaheliotropism of radial members. Annals of Botany. **10** : 294. 1896.
[2] Day, R. N. The forces determining the positions of dorsiventral leaves. Minnesota Botanical Studies, **1** : 743. 1894–98.

dow through which a strong light is received. Continue the
motion of the clinostat until the leaves have attained average

size, for which several days
will be necessary, and note
the position of the leaflets.
Now place a second plant on
the clinostat in the same man-
ner, but set the apparatus to
direct the axis of the plant
parallel to the window and
note the final positions of the
leaflets.

212. Compass Plants.
Leaves exposed to sunlight
in a horizontal position receive
the rays of a noonday sun

FIG. 70. *Arisaema triphyllum* rotated
on horizontal axis parallel to window.

at right angles, and are thus subjected to the maximum intensity
of illumination. Large numbers of species avoid such intensities
by growing only in shaded habitats. A few forms attain a similar
end by placing the leaves with the edges vertical and directed
north and south, on which account they have become known as
" compass plants." A leaf in this position receives two maxima
of illumination daily, one in mid-forenoon and one in mid-after-
noon, but these maxima are far below the maximum to which a
horizontal leaf is exposed.

Grow a number of *Lactuca* in the open air where they may
receive sunlight during the entire day and note the position of
the leaves. Grow a similar number under the shade of a tree
or a building, and compare the results. Note the torsions nec-
essary to carry the leaves to their positions. Other compass
plants are *Silphium laciniatum* and *Wyethia*.

213. Other Reactions due to Intensity of Illumination. The
varying intensity of light has been the cause to which may be
ascribed several adaptations on the part of the plant by which
the injurious exposure may be avoided. Chief among these are

the photolytic movements of chloroplasts in exposed cells, para-phototropic reactions, and photeolic, or nyctitropic movements

FIG. 71. *Lactuca scariola* (compass plant), seen from east or west. The leaves have been moved by curvatures and torsions until the laminae are directed approximately north and south to within a few degrees. After Atkinson.

of leaves. The chloroplasts of a large number of species move toward the walls parallel to the surface of the leaf in diffuse light,

and to the walls at right angles to these in strong illumination as in Fig. 72, although some investigators deny the economy of such movement. These movements may be seen in leaves of *Oxalis* placed in various intensities of light and there sectioned.

214. Paraphototropism. A large number of species have the power of changing the positions of the laminae in such manner that the angle at which rays strike the surfaces are varied with the intensity. By reason of this adaptation many forms exhibit movements during the intense illumination of midday, which are termed paraphototropic movements. Generally such movements consist in reactions resulting in directing the apices of the leaves or leaflets toward or away from the source of illumination. If the leaves of almost any leguminous plant are examined at noon on a hot summer day, or in a tropical greenhouse these positions may be observed. *Trifolium*, *Mimosa*, *Cassia*, *Oxalis*, *Phaseolus*, and others are good objects for these observations.
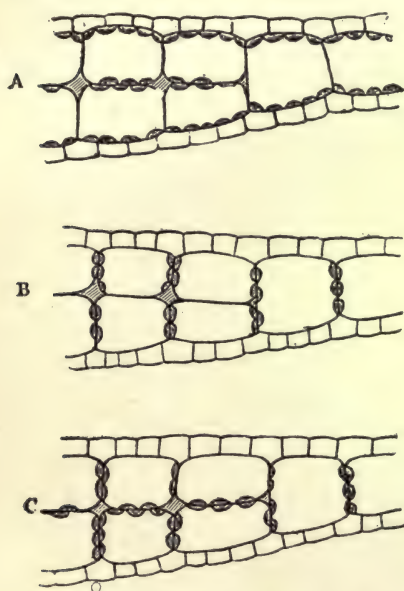
FIG. 72. Positions assumed by chloroplast in *Lemna trisulca*; *A*, in diffuse light, *B*, strong diffuse light and *C*, in direct sunlight. After Stahl.

215. Nyctitropic Movements. Note the positions of the leaflets of any of the leguminous species mentioned in the last experiment after 5 P. M. and early in the morning. The positions assumed are much more marked than those of the paraphototropic reactions and may consist in moving the laminae downward or upward. The nyctitropic movements seem to be very clearly due to differences in illumination, although the reaction is

FIG. 73. *Mimosa pudica.*
Normal position of leaves.

FIG. 74. *Mimosa pudica.*
Noonday position of leaves.

FIG. 75. *Mimosa pudica.*
Position of leaves at night.

directly concerned with low tempera-
ture, and rapid radiation of heat from
the tissues of the plant. Plants have
been subjected to the diurnal fluctua-
tions of light and temperature so long
that a rhythm of action is set up that
persists for several days, even when
the plant is placed in continuous dark-
ness or light.[1]

216. Formative Influence of Light.
Light has a most notable influence
in the determination of the external
form of a large number of plants.
One phase of this action has al-
ready been discussed under *Etiola-
tion* in which the reactions of the
plant in total darkness are discus-
sed. The dorsiventrality of prothal-
lia, and shoots in general is due to the
action of this agency. The develop-
ment of certain tissues or organs on
one side of the axis of a shoot, and
their suppression on other parts of
the body, may be regulated by illu-
mination which, in consequence, gen-
erally flattens and increases the sur-
faces devoted to the exposure of
chlorophyl. Different developmental
stages of an organism find their opti-
mum in different intensities of illu-
mination, and if these are not fur-

[1] Jost, L. Ueber die Abhängigkeit des Laubblättes von seiner Assimilations-
thätigkeit. Jahrb. Wiss. Bot. **27** : 403. 1895.
Jost, L. Beiträge zur Kenntniss der nyctitropischen Bewegungen. Jahrb. Wiss.
Bot. **31** : 345.

nished, changes in function as well as many other alterations affecting the general physiology and organography of the plant ensue.[1]

217. Production of Primordial Leaf-forms by Diffuse Light.
Campanula rotundifolia forms two kinds of leaves, one rounded, and cordate at base, on the lower part of the stem in the earlier stages

Fig. 76. *Cassia* (a tropical species) showing normal position of leaves in moderate illumination.

of its development, and the other lanceolate or linear, in the later stages. The first are developed from the stem when it is usually more or less excluded from the light by the loose substratum, while the latter are developed in the full illumination of sunlight. To demonstrate this action establish a dozen healthy plants in suitable pots in the spring and place them in a row, beginning near a window and extending into a part of the room in which only

[1] For full discussion of this subject see Goebel, K., Organography of Plants, 227. 1900.

a weak diffuse light penetrates, and keep under proper cultural conditions for a month.   Care must be taken to maintain the plants in healthy green condition, since the reaction is in no sense an etiolative one.   The main shoot bearing flower buds will probably not develop anything but normal upper leaves, but the lateral branches of the shaded specimens will show rounded leaves of the types usually formed at the bases of the stem.

FIG. 77.   *Cassia* at noonday with intense illumination and high temperature.
FIG. 78.   *Cassia* (see Fig. 75) showing position of leaves at 6 P. M. (sunset) in warm room.

218. Influence of Light on the Formation of Tubers.   The thickenings of the stolons arising from the basal portions of the main axes of plants of *Solanum tuberosum* (potato) form the well known potatoes.   These formations generally arise only in organs from which the light is excluded, although known to occur in plants growing closely crowded together and hence shaded from intense light.   Fill a few flower pots completely full of

rich garden soil, and set upright in the center of each a large sound potato of some " early " variety in such manner that the upper end is exposed.   Place in a temperate room under diffuse light and water sparingly.   After a time the germination of the tuber will produce several stems from the different buds. All of these should be destroyed except one on each tuber.   This will develop a main axis, and stolons from the basal part of the main axis.   After the stolons have attained a length of 10 or 15 cm. the main axis is cut away and one of the stolons is raised and its end thrust into a small dark chamber consisting of a zinc or cardboard box which may be tightly closed.   This box should be about 15 cm. by 11 cm. and one end should be slit in such a manner that the stolon may be introduced sidewise, the slit closed with cotton wool to exclude light and then the lid put in

FIG. 79.   Shoot of *Campanula*, showing rounded leaves developed on upper lateral branch at *A*.   After Goebel.

place.   It will be still better to have the box made of zinc and the lid replaced by a slide.   The box should exclude all light, and should be shaded by cloth from the direct rays of the sun. In order to receive the moisture accumulating in such enclosed spaces containing transpiring shoots, a small vessel containing sulphuric acid may be set inside the box.   A stand or support will serve to hold the box and other parts of the preparation in place.   The box should be opened from time to time to take away the etiolated leaves which quickly turn yellow and die. The formation of tubers may be soon noticed, and their growth will be extensive inside of a month. [1]

[1] Vöchting, H.   Ueber die Bilding der Knollen.   Bibl. Bot.   Hft., 4.   1887.

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If a large dark chamber is at hand various methods of treatment may be used, in which the main axis (fore-shoot) only may

Fig. 80.   Stolon of *Solanum* extending into a small dark chamber in which a branch has been convérted into a tuber.   After Vöchting.

be allowed to develop, producing a club-shaped stem 15 to 25 cm. long and 1 and 2 cm. in thickness, with similarly thickened branches at the apex : or all of the stems and branches of a bud may be allowed to remain, with results of value in the analysis of the factors operative in the formation of tubers.

IX. COMPOSITION OF THE BODY [1]

219. Substances Found in Plants. The principal components of living matter are carbon, hydrogen, oxygen and nitrogen, while a few other elements play more or less minor parts in the plasmatic structures. The compounds found on analysis of the body of a plant comprise both the components of the living matter, and also the substances which have been formed by it and deposited in the form of secretions retained in the body, in the cells, or in the form of dead tissues, which serve mechanical uses only. These compounds are proteids, amides, alkaloids, carbohydrates, organic acids, glucosides, fats and fixed oils, and essential or volatile oils, etc., but this enumeration does not give a defined basis from which the study of the metabolism of the plant may proceed until a differentiation is made between the substances participating further in the activities of living matter, and those in which no further change is possible. The former which may be known as *plastic* substances include starch, sugar, inulin, glycogen, cellulose, globulin, amides, fats, oils, glucosides, organic acids, and many others, while the latter or *aplastic* substances comprise the cellulose of the walls of dead tissues, insoluble crystals of mineral salts, waxy substances, etc. It is to be noted that many substances participate in the construction of both kinds of material. Thus cellulose is in most instances an aplastic substance, but when deposited as reserve food in the seeds of Liliaceae and other plants, it is plastic.

The outlines of analysis on the following pages will give methods for the detection and estimation of the more important substances which may be extracted from plants.[2]

[1] The draft of this chapter was prepared by Mr. J. E. Kirkwood, and Dr. W. J. Gies, who also read proof of the pages.

[2] For the identification of these substances in the tissues, the methods of microchemical analysis given in Zimmermann's Botanical Microtechnique will be necessary.

220. Carbohydrates. The carbohydrates are non-nitrogenous bodies of various degrees of stability, differing much in physical and chemical properties, and consisting of carbon, oxygen and hydrogen. The carbohydrate molecule usually contains six or a multiple of six atoms of carbon, while the hydrogen and oxygen are present in the same proportion as in water, with at least five atoms of oxygen to six of carbon.[1]

Carbohydrates are neutral in reaction and combine loosely with other bodies, especially bases. The following properties are characteristic of the greater number of these substances :

(*a*) They reduce alkaline metallic solutions and are colored yellow by alkalies.

(*b*) They rotate the plane of polarized light.

(*c*) They give characteristic crystals with phenyl-hydrazine.

(*d*) Most of them in contact with yeast are broken down into alcohol and carbon dioxide, *i. e.*, they are fermentable.

(*e*) They give color reactions with acids and aromatic alcohols.

(*f*) They are mostly soluble in water. Those which are not, can be dissolved by heating with an acid in which process they are hydrated into soluble sugars, however.

Most of the carbohydrates may be classified as follows :

I. Glucoses or Monosaccharids, $C_6H_{12}O_6$.
+ Dextrose.[2]
— Levulose.
+ Galactose.

II. Saccharoses or Disaccharids, $C_{12}H_{22}O_{11}$.
+ Cane-sugar.
+ Lactose.
+ Maltose.
+ Iso-maltose.

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[1] There are some exceptions to this rule, such as bioses, trioses, tetroses, etc., in which the carbon is present in two, three, and four atoms respectively, and also such as rhamnose, which has twelve atoms of hydrogen to five of oxygen.

[2] The signs + or — indicate that the more familiar of these substances when in solution rotate the plane of polarized light to the right or left respectively.

III. Amyloses or Polysacchariads, $n(C_6H_{10}O_5)$.
  + Starch (paste).
  + Dextrin.
  + Glycogen.
    Cellulose (insoluble in water).

The sugars of the first class are characterized by the readiness with which they take up oxygen from their surroundings and thus reduce bodies rich in oxygen. Upon this fact depend some of the most important tests for their recognition, viz., the reduction of alkaline metallic solutions. The monosacchariads are further characterized by their susceptibility to the action of yeast-cells, being broken down by the enzymes of these organisms into alcohol and carbon dioxide.

The disacchariads do not all reduce alkaline metallic solutions. They may, however, be transformed into monosacchariads by boiling with dilute acids. They thus undergo a hydrolytic cleavage, commonly termed inversion, in which process they are transformed into glucoses. Two molecules of a monosaccharid can be obtained from one hydrated molecule of a disaccharid. Their relations may be shown thus :

Cane-sugar + H_2O = dextrose + levulose
Maltose   + H_2O = dextrose + dextrose.

It will be observed that the sugars are included in the first two classes : they are either glucoses or saccharoses. The third class, or amyloses, may be regarded as the anhydrides of the glucoses. They are called polysacchariads because their molecules are made up of a multiple number of glucose molecules minus an equal number of molecules of water $[n(C_6H_{12}O_6) - n(H_2O) = n(C_6H_{10}O_5)]$. Glucoses may be obtained from some of the amyloses by hydrolysis, accomplished either by boiling with dilute acids or by the action of an enzyme. In the formation of dextrose from starch various intermediate products appear, such as soluble starch (amylodextrin), different varieties of dextrin, maltose and isomaltose. Most of those celluloses which occur in the cell walls of the ordi-

nary tissues of plants offer some resistance to hydrolysis, but the cellulose which is stored in the endosperm of seeds is capable of being decomposed by acids with the formation of carbohydrates of relatively low molecular weight.   Thus carbohydrates are plastic substances capable of being transformed from monosaccharids to disaccharids and polysaccharids and vice versa.   As these changes are continually going on in the natural processes of metabolism, the analysis of any plant would probably reveal carbohydrates of different degrees of complexity.   The sugars commonly occur in solution in the sap of various plants, although they may sometimes be found in crystalline form, as in certain sacchariferous seeds.   The starches are found most abundantly in tubers, roots and seeds, while the celluloses, pentosans, lignoses, etc., form the principal part of the framework of the plant.

221. Fractional Extractions.   The following extractions make a preliminary separation of the carbohydrates into groups, and separate them from other matter in such manner as to leave them free for determination or estimation.   The material should be very finely divided and air-dried.

I. EXTRACTION WITH BENZINE.   Extraction with benzine removes the oils, fats, resins, pigments, etc. The operation should be made at the boiling point (not above 75 ° C.) over a steam bath and away from a flame.   The Soxhlet apparatus is by far the best

FIG. 81.
Soxhlet's apparatus for extraction with benzine and alcohol

and most economical for the purpose.   When nothing further can be extracted by a fresh quantity of the solvent, the extraction may be considered complete. When this is accomplished the residue should be dried at 100° C. and weighed.

II. EXTRACTION WITH ALCOHOL.   The dry residue from I. should next be extracted in the same manner with boiling alcohol of 0.85 sp. gr.   This process removes tannins, glucosides and part of the sugars.

III. EXTRACTION WITH DILUTE ACID, OR DIGESTION WITH MALT.   The material from II. should be washed well with water and treated with 1 per-cent. sulphuric acid at a temperature of 100° C.

In this extract are the products of the hydrolysis of starch, disacchorids and other carbohydrates.   The extraction should be continued until the solution fails to give any reaction with iodine. Reducing sugars with possibly a small quantity of dextrin should be the final product.   If erythrodextrin is present it will be indicated by a red coloration upon the addition of iodine, but achro-odextrin, which is a later stage in the hydrolysis of starch, gives no color with that reagent.

Instead of boiling with sulphuric acid, the same results may be obtained by digesting the residue with a solution of malt diastase, at a temperature not to exceed 60° C.

The malt extract may be made by extracting 50 grams of pulverized malt, which has been dried at a low temperature, with 300 cc. of water.   100 cc. of this solution should be used with every 10 grams of the material to be digested.   As in the case of the extraction with acid, the same tests should be applied to determine the end of the digestion.

222. Estimation of Tannins and Glucosides.   Tannins are slightly acid, amorphous, colorless substances, soluble in alcohol, ether, insoluble in benzine, benzol, chloroform, and oils, giving blue or green precipitates with salts of iron.   All are precipitated by gelatin or albumin.   Tannic acid is the principal constituent of tannins and it occurs almost pure in nut-galls.

The alcoholic extract (II.) should be evaporated and the residue dissolved in water and filtered.   The reaction to litmus should be tested and if the solution is acid should be carefully neutralized with dilute sodium carbonate.

Test portions of the neutral solution with the following substances :

1. Solution of gelatine.   Tannins indicated by dirty white precipitate.

2. A few drops of ferric chlorid or ferric acetate. Blue or green precipitate shows tannins.

3. Ammoniacal solution of potassium ferricyanide. Presence of tannins indicated by deep red color changing to brown.

4. Lime-water, $Ca(OH)_2$. Tannins shown by blue, brown or red color or precipitate.

QUANTITATIVE DETERMINATION OF TANNINS.   Only one method will be given here.   If others are desired see Wiley, Principles and Practice of Agricultural Analysis, Vol. III.   1897.

Two and a half grams of gelatine are dissolved in water with ten grams of alum, and the solution made up to one liter.   This solution and the solution of tannins are both heated to 70°C.   The gelatine solution is then slowly added with constant stirring to the solution of tannins.   Continue to add the gelatine until the pre-cipitate coagulates, and until the further addition of the reagent causes no additional precipitation.   Collect the precipitate, dry at 110°C and weigh.   Fifty-four per cent. of the weight of the pre-cipitate is pure tannin.

TESTS FOR PHLOROGLUCIN.—If the material extracted was woody tissue phloroglucin may be present in the extract.   As this substance is soluble in ether, it may be removed by shaking ether with the extract.   The ether should be separated from the aqueous solution by means of a separatory funnel and evaporated, and the residue dissolved in water.   To parts of this solution add :

1. Hydrochloric acid and vanillin.   Phloroglucin is indicated by a reddish-violet color.

2. Ferric chloride.   A deep violet color will result if phloro-glucin is present.

223. Determination of Sugars and Dextrins.   Finely ground tissue should be extracted with ether, or benzine, to remove fats, etc., and after being freed from the ether by drying in the air, the residue should be extracted with water near the boiling point for about an hour.   The mass should then be thrown on the filter and the filtrate treated with basic lead acetate to remove as much as possible of the proteids.   After filtering out any precipitated

proteids and removing all traces of lead from the filtrate with a current of H_2S, the filtrate should be treated with gelatine to remove tannins by the method already given, and the solution filtered clear and the filtrate preserved. A little chloroform should be added as an antiseptic if the solution is not to be examined immediately.

A minute examination of the filtrate would probably reveal several different carbohydrates. For the present purpose it will be sufficient to examine it for glucoses, maltose, cane-sugar, dextrins and inulin.

The direct and accurate determination of the quantities of different sugars and dextrins in the same solution is hardly possible by any of the methods now known. It is obvious that the polariscope cannot be depended upon entirely to identify any particular substance when there are several optically active in the same solution. Most of the precipitants usually employed do not exercise sufficient selectivity, and carbohydrates of different classes are carried down together. Fermentation processes are hardly more satisfactory, inasmuch as yeast not only decomposes glucoses, but inverts and subsequently breaks down the disaccharids as well. It will be possible, however, to outline some qualitative determinations which will enable one to identify those sugars and dextrins most commonly occurring in plants.

The aqueous solution should be divided into several parts. One part is evaporated to a syrup and about ten volumes of 99 per-cent. alcohol added. After stirring well the precipitate is collected on a filter, washed thoroughly with alcohol of the grade noted above and dried. This precipitate may be considered mostly dextrin, though it is probable that some reducing sugar may be present also. The weight of the dry precipitate may be taken and some idea gained of its proportion to the other substances. The precipitate should then be dissolved in water and a part of this solution tested with iodine. A red coloration will indicate erythrodextrin, blue will indicate amylodextrin. The color should disappear on heating and reappear on cooling.

To a portion of the solution add caustic soda. No color is obtained with iodine. In solution dextrins are not precipitated by basic lead acetate alone, but by basic lead acetate and ammonia.

A portion of the solution of the alcoholic precipitate may here be tested for inulin.

Add to some of the solution in a test-tube a few drops of strong hydrochloric acid and boil. Cool the solution and add a few drops of phloroglucin in alcohol. The presence of inulin is indicated by a yellow-brown color.

To another part of the solution add baryta water until no further precipitate is formed. After washing the precipitate, decompose it in water with a current of CO_2, filter and evaporate the filtrate ; if inulin was present crystals should be obtained.

After the precipitation of the dextrins the alcoholic filtrate would probably contain most of the sugars, possibly glucoses, maltose and cane-sugar. This filtrate should be evaporated to remove all the alcohol and the residue dissolved in water. Test portions of this solution with the following reagents :

1. Fehling's solution. Fehling's solution should be first tested by boiling a little in a test-tube. If red cuprous oxide is not precipitated by this treatment the solution is still good. Add some of the extract and boil. A greenish coloration followed possibly by a yellow precipitate, which finally turns red, indicates the presence of a reducing sugar.

Fehling's solution should be kept in two parts to ensure its preservation. Solution A should consist of 34.64 grams of pure cupric sulphate dissolved in 500 cc. of distilled water. Solution B should be made up of 173 grams of Rochelle salts (sodio-potassium tartrate) in 100 cc. of pure caustic soda sp. gr. 1.34, and water to 500 cc. When ready to use mix equal volumes of A and B.

2. Barfoed's solution. This solution gives a precipitate of red cuprous oxide on boiling, if dextrose is present. Lactose, maltose, cane-sugar, and dextrin when heated with it for a short time give no reaction.

Barfoed's solution is made by taking 200 cc. of a solution of neutral acetate of copper, containing one part of the salt to 15 of water and adding to it 5 cc. of a 38 per-cent. solution of acetic acid.

3. Cobaltous nitrate (five per-cent. solution).   Add 5 cc. of cobaltous nitrate solution to about 15 cc. of the solution to be tested.   After the solutions have been well mixed add 2 cc. of a 50 per-cent. solution of sodium hydrate.   With this reagent cane-sugar will give a permanent amethyst violet color.   Dextrose gives a turquoise blue color but in a mixture of the two sugars the cane-sugar color reaction is predominant, and can be detected though the cane-sugar may not form more than one tenth of the mixture.   The cane-sugar coloration on boiling turns slightly bluish, but is restored to its original condition on cooling.   In a few hours the color given by dextrose will change to pale green. Maltose gives about the same color as dextrose, though not so fine a green color at last.

4. Phenyl-hydrazine hydrochloride.   To about 10 cc. of the sugar solution in a test-tube add two parts of phenyl-hydrazine hydrochloride and three parts of sodium acetate.   Keep in boiling water in the water-bath for an hour and a half and then place the tube in cold water.   Examine the crystals under a microscope.   Dextrose, levulose, maltose and galactose form osazones with phenyl-hydrazine, but cane-sugar does not.   Galactose is very rare in plants and the osazone of levulose has the same properties as that of dextrose.   To separate roughly these two osazones allow the tube containing the mixture to stand in the cold several hours, and finally filter.

Maltosazone is quite soluble in cold water and will appear in the filtrate upon evaporation to a small volume, while the dextrosazone will remain in the solid state.   The maltosazone should be purified by dissolving it again in water and reprecipitating it by alcohol. The sugars from which the osazones come can be better identified by the melting points of their phenyl-hydrazine compounds. Maltosazone melts at 206° C., Dextrosazone at 204°–205° C.

5. Amount of fermentable sugar in the solution.   Fill an Ein-
horn's saccharimeter (See Fig. 82) with the solution after a little

compressed yeast has been shaken up
in it, taking care to fill the graduated
limb of the instrument.   The yeast must
be active and free from fermentable car-
bohydrates.   Set the instrument in a
warm place.   After fermentation has
ceased the amount of CO_2 evolved is
read off on the graduated scale.   The
figures will indicate directly the amount
of fermentable sugar in the solution.   A
control test should be made by taking a
second instrument of the same kind and
introducing water and some of the same
yeast.   The amount of carbon dioxide
evolved in this way should be subtracted from the quantity in the
other instrument.

FIG. 82.   Einhorn's fermen-
tation saccharimeter.

6. Crystals of cane-sugar.   Add
strontia-water to the solution in con-
siderable quantity, and after filter-
ing, evaporate the filtrate until a
yellow amorphous precipitate begins
to separate out.   After it has stood
for some time collect the precipitate
and add to it dilute alcohol and de-

FIG. 83.   Crystals of cane-sugar.

compose it with carbon dioxide.   Filter this solution and re-
duce the solution somewhat by evaporation.   Add 95 per-cent.
alcohol until a precipitate begins to form ; add a crystal of cane-
sugar to induce general crystallization and allow to stand.   Cane-
sugar crystals are characteristic in form (See Fig. 83).
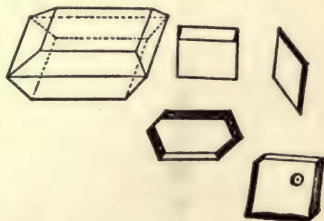
If the above experiments indicate the presence of cane-sugar
and glucoses in the solution, the remainder of the extract should
be divided into two equal parts and treated as follows :

1. Determine the quantity of reducing sugar (glucoses and

maltoses) in the mixture.   Of pure dextrose 0.5 gm. in 1 per-cent. solution reduces 101.1 cc. of Fehling's solution diluted with four volumes of water.   But as we are not dealing with a pure dextrose the results can only be approximate.   To a known quantity of dilute Fehling's solution boiling, add from a burette the sugar solution drop by drop.   When just sufficient has been added to cause the last trace of blue color to disappear from the boiling mixture the reduction may be considered complete.   If maltose was present in the solution it also acted upon the Fehling's solution and it would be impossible to calculate directly the amount of dextrose. But if experiment 4 revealed no maltose the dextrose may be estimated from the data given above.

2. To the other half of the solution add enough hydrochloric acid to make a 2 per-cent. solution.   Heat on the water-bath for two or three hours, cool and neutralize.   Determine the reducing power of this solution.   The difference between this and the foregoing determination will give the amount of invert sugar formed.[1]

224. Starch.   Starch is capable of being separated from the tissues in which it occurs by grinding the tissue to a pulp and washing on a coarse cloth.   The starch is carried through with the water, and if this is allowed to flow into a tall jar or cylinder it will settle to the bottom, and may be washed and separated by repeated accession and decanting of water.   The following tests will be found the most useful.

1. Rub a gram of starch with cold water in a mortar, and stir the paste into 50 cc. of boiling water.   An opalescent, imperfect solution is obtained.   Starch is only slightly soluble even in hot water .

2. To a little of the starch solution add iodine solution.   A deep blue color appears, which disappears on heating, and reappears on cooling, if not boiled too long.

[1] Maquenne, L.   Les Sucres et leurs Principaux Dérivés.   Paris.   1900.
Stirling, Wm.   Practical Physiology.   Philadelphia.   1898.
Verworn, Max.   General Physiology.   London.   1899.
Darwin and Acton.   Physiology of Plants.   Cambridge.   1894.
Rijn, J. J. L. van.   Die Glykoside.   Berlin.   1900.

3. Add a little Fehling's solution to the starch solution and boil. No reduction.

4. Boil some of the solution with a little dilute hydrochloric acid for half an hour. As soon as the solution begins to clear soluble starch is formed. Draw off a little of this and test with iodine solution. Amylodextrin (soluble starch) gives a blue color. A little later try the same reaction. A red color indicates erythrodextrin. When no coloration follows the addition of iodine the starch may all be considered inverted although some achroodextrin may still be present. Boil some of the solution, neutralized with sodium carbonate, with Fehling's solution. A reduction of the solution follows.

5. Add to 20 cc. of the solution 10 cc. of malt diastase solution (See page 151) and try the same reactions as in 4.

225. Cellulose. Cellulose, like starch, is insoluble in water and all the weaker solvents. Some reagents dissolve it, and from these solutions it may be precipitated practically unchanged. It occurs in all the tissues of the higher plants.

1. It is soluble in ammoniacal solution of cupric oxide and is precipitated from this solution by acids. Try dilute hydrochloric.

2. Cellulose gives a blue color with sulphuric acid and iodine, but no color with iodine alone.

3. Dissolve some cellulose in a concentrated acid. Add water and note the gelatinous precipitate, which is called **amyloid** and gives a blue color with iodine.

4. DETERMINATION OF CELLULOSE. Extract a few grams of tissue with ether. After removing the ether thoroughly from the residue by drying at a temperature below 40° C., place the residual substance in a hard glass beaker with 200 cc. of boiling 1.25 per-cent. sulphuric acid. Cover the beaker with a watch glass and continue boiling for thirty minutes. Wash the tissue on a filter with hot water until all the acid is removed. Return the material to the same beaker and add 200 cc. of boiling sodium hydrate. Continue the boiling for thirty minutes, wash the tissue free of alkali, dry, weigh and incinerate. The loss of weight after

incineration is regarded as the quantity of cellulose. The solutions used should be exactly of the strength specified, and the sodium hydrate pure.[1]

226. Proteids. The various proteids differ much in their elementary composition, but the percentages of the elements average about as follows :

,	C	H	N	O	S	P
	52	7	16	22	0.5–2	0.3–1.5

The constitutional formula of proteids is as yet unknown.

Proteids have the following properties in general :

(*a*) They are all insoluble in ether, and most of them insoluble in alcohol. Most proteids are soluble in water. Other more general solvents are dilute and concentrated saline solutions, weak acids and weak alkalies. They are all decomposed by the action of concentrated mineral acids or alkalies and by the action of certain enzymes.

(*b*) Some proteids when in solution are coagulated by heat.

(*c*) Proteids (with the exception of hydrated varieties, such as proteoses and peptones) are indiffusible ; that is, they are incapable of passing through dead animal membranes.

(*d*) All proteids in solution are laevo-rotatory.

(*e*) With certain mineral reagents they give characteristic color reactions.

(*f*) Most proteids are precipitated by salts of the heavy metals, by picric acid, by acetic acid and potassium ferro-cyanide, by saturation with certain neutral salts, as ammonium sulphate and by strong acids.

Proteids are divided with reference to their origin into two classes, animal and vegetable, and as in both cases they are often found combined with other bodies, they are further classified on this basis as simple or compound. It may be said here that the compound proteids offer many exceptions to the general proper-

[1] Wiley, H. W. Principles and Practice of Agricultural Analysis. 3 : 1897.
Hammarsten-Mandel. Text-Book of Physiological Chemistry. 1899.

ties given above. A third class, the albuminoids are, in general, particularly insoluble.

The simple vegetable proteids may be divided roughly into six classes according to their varying solubilities.

I. Albumins. These are proteids soluble in water. They are not precipitated from solution by sodium chloride, magnesium sulphate or acetic acid. They are coagulated by heat at from 65° to 70° C. Leucosin from barley is an example of a vegetable albumin.

II. Globulins. This proteid is insoluble in water, soluble in a dilute solution of sodium chloride, but is partly or completely precipitated by saturation with the same salt. They are of two kinds, myosins and paraglobulins. The former coagulate at 55°– 60° C., but the latter at a higher temperature, 70°–75° C. Of this class are tuberin (from the potato), vitellin (from maize), and edestin which is found in many seeds.

III. Albuminates (acid or alkali albumin), are soluble in dilute acids or dilute alkalies, and precipitated from such solution by neutralization. They are insoluble in water or neutral saline solutions. The principal vegetable albuminates are conglutin and legumin. The latter is a " vegetable casein " and occurs mostly in leguminous seeds.

IV. Coagulated proteids are proteids that have been made less soluble by heat, or chemical reagents, such as alcohol, or ferments. They are hydrolyzed and dissolved by proteolytic ferments. They are insoluble in water, saline solutions, dilute acids and dilute alkalies.

V. Proteoses. This term includes albumoses (from albumin), globuloses (from globulin), etc. These bodies are intermediate products in the hydrolysis of proteids. They are soluble in water, saline solutions and acids ; they are precipitated by satura- tion at boiling temperature with neutral ammonium sulphate. They are non-coagulable. The vegetable proteoses are called phyto-proteoses.

VI. Peptones. These are the final products of the hydration

of proteids. They are exceedingly soluble in water and are not precipitated by sodium chloride, acids or alkalies. They are precipitated by tannic acid, but not by ammonium sulphate. They are not coagulated by heat. Germinating seeds often furnish a large percentage of peptone.

Inasmuch as proteids are plastic substances and are constantly undergoing tranformation in the metabolism of the plant, a mixture of proteids will generally be found in the analysis of any vegetable organism in various stages of hydrolytic change. Proteids occur to the greatest extent as reserve foods in the various specialized parts of plants such as tubers, roots and seeds, and very often are found in solid form especially in the cereals.

227. Extraction of Proteids. The material to be extracted should be very finely divided and then treated first with benzine to remove the bulk of fats, pigments, etc. It is well to follow this treatment with the same quantity of 95 per-cent. alcohol, for a few hours and lastly extract with pure sulphuric ether. In the case of certain cereals in which the quantity of fat or oil is practically nothing, this process may be omitted.

The extractions should all be carried on at a temperature not to exceed 35° C. in order to avoid coagulating any of the proteids present. The last traces of ether may be removed by spreading out the tissue in shallow dishes and allowing it to dry at room temperature.

The material should now be covered with at least twice its volume of dilute solution of common salt (about 10 per-cent.) and the extraction allowed to continue with repeated stirring until all the proteids soluble in such a solution are removed. This may be determined by adding fresh solvent from day to day. The extracts which are drawn off are preserved separately, and a little powdered thymol added to each to preserve from mould.

228. Separation of Proteids. The extract should be filtered and the filtrate dialyzed. For this purpose the solution is placed in a bag of vegetable parchment and suspended in running water. The dialysis should be continued for several days, or until the

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chloride is entirely removed.   If a little of the solution is drawn off from time to time and a drop of silver nitrate added with a little dilute HNO_3, the presence of chlorides can be detected by a white precipitate.   After the salt has entirely disappeared from the dialyzing bag its contents can be removed and examined.   As globulins are insoluble in water any precipitate will probably be a proteid of that class.   Sometimes organic matter other than proteids is separated out by dialysis from a saline solution.   If a precipitate is present it should be collected on a filter, and the following tests performed :

229. General Qualitative Tests for Proteids.   1. Examine the precipitate under the microscope.   Globulins frequently separate out in various crystalline forms.

2. To a little of the precipitate in a test-tube add caustic potash solution, and afterwards a trace of copper sulphate in very dilute solution.   A reddish to violet color more or less distinct depending upon the quantity of the proteid and copper present is to be seen.   This is called the biuret reaction.

3. Boil a little of the material in concentrated nitric acid.   Cool the liquid by holding the test-tube under the water flowing from the tap for a minute or two and add ammonia.   If proteids are present a yellow color will be imparted to the nitric acid on boiling, which changes to orange, upon the admixture of ammonia. This is the xanthoproteic reaction.

4. Add Millon's reagent and heat gently at first, but if no reaction is apparent, bring the liquid to the boiling point, when a brick red color in the precipitate will indicate the presence of proteids.   If traces only are present the color will be produced in the solution.   Chloride tends to vitiate the test, by combining with the mercury in the reagent.

5. Dissolve some of the precipitate in a saline solution as dilute as may serve the purpose.   Boil some of the solution in a test-tube and then add a drop of dilute acetic acid.   A precipitate should occur if globulins are present.   The acid must not be added before the boiling point is reached.

6. If a coagulable proteid is present its temperature of coagulation should be determined in the following manner : Some of the solution is placed in a test-tube in which is inserted a thermometer. Suspend the test-tube in a beaker of water, and set this beaker in a second containing an amount of water sufficient to reach the level of the first and heat very gradually over a Bunsen flame. The temperature at which turbidity is at first noticeable and also the point at which the precipitation becomes flocculent should be carefully noted.   The globulins vary greatly in their coagulation temperatures, but usually do not fall below 55° C.   After the first flocculent precipitate is obtained by this method remove it by filtering and return the filtrate to the water-bath.   Raise the temperature as before and note any further changes.   It is possible to make several such fractional coagulations at successively higher temperatures in solutions in which several proteid substances are present.

7. Treat a portion of the solution of the precipitate from the dialyzer, with solid magnesium sulphate in excess, which will precipitate globulins.

If the above treatment indicates that the substance is a proteid it should be washed thoroughly, first with alcohol, and then with ether and dried in a thermostat to a constant weight at 110° C., or at room temperature over sulphuric acid.

Other substances are often thrown down with the globulins upon dialysis, and an effort should be made to obtain the proteid as pure as possible.   This may be done in various ways.   Dissolving the proteid in a saline solution as dilute as may serve, filtering and repeatedly dialyzing may accomplish the desired result. Dissolving the proteid in the smallest amount of saline solution that would serve, filtering and adding to the filtrate a larger volume of distilled water, will often precipitate the globulins in pure form.

It is usually necessary to determine the percentage of the elements, especially the nitrogen contained in order to identify any globulin.   The total proteid of a tissue can be approximately es-

timated by determining the total nitrogen content and multiplying the result by the factor 6.25. The determination of the nitrogen should be done in accordance with the Kjeldahl method, which is described in nearly all works on volumetric analysis.

230. Tests for Albumin. The filtrate from the material from the dialyzers may now be examined. It may contain albumin, proteose and peptone, as these substances are all soluble in water, and should not be precipitated by the removal of the salt.

1. Try the color reactions (page 162) on portions of the fluid.

2. Coagulation test. Apply as directed for globulin. Vegetable albumins coagulate at about 65–70° C. Heat the remainder of the filtrate until the coagulable proteid is all thrown down. Collect the coagulum on a filter, wash with alcohol and ether, and dry as directed for globulin. The albumin will undergo decided changes during this process, and it will be profitable to note some of its properties as coagulated proteid.

(*a*) Test solubility in water, dilute alkalies and dilute acids.

(*b*) Boil for some time with very dilute acid or alkali. It should be dissolved slowly.

(*c*) Test with strong acid or alkali. It is quickly decomposed.

(*d*) Test it with a solution of pepsin to which are added a few drops of hydrochloric acid. Keep at a temperature of 40° C. The coagulated proteid becomes hydrolyzed by the enzyme and is dissolved. Filter the solution and neutralize with sodium hydrate. Acid albuminate is precipitated, unless all has been transformed to proteoses and peptones.

231. Treatment of Proteoses. The filtrate obtained in the separation of the coagulum may contain proteoses and peptones. Test the fluid for proteoses by the color reactions. If positive results are obtained saturate the filtrate at boiling point with ammonium sulphate, which will precipitate all proteoses. The peptones still remain in solution. The precipitates having been removed by filtering and dissolving in water the ammonium sulphate may be removed by dialysis, or by the addition of barium

carbonate and warming on the water-bath. In the latter case the sulphate is precipitated as barium sulphate, and the ammonia may be driven off by heat, leaving the proteoses in solution. The proteose may be precipitated from the concentrated solution by filtering it directly into alcohol, from which it may be separated by filtration. It may be dried as in the preparation of the other proteids. Make the following tests upon the proteids in solution :

1. The biuret test gives a rose-red coloration.

2. Try the following precipitants and notice that the precipitate disappears on heating and reappears on cooling in every case.

(*a*) Picric acid.

(*b*) Potassio-mercuric-iodide, and hydrochloric acid.

(*c*) Trichloracetic acid.

(*d*) Acetic acid and saturation with sodium chloride.

232. Tests for Peptones. The filtrate obtained in the separation of the proteoses should be extracted once or twice with one-fifth volume of 95 per-cent. alcohol, and the alcoholic solutions after filtration and concentration should be treated with barium carbonate for the purpose of removing the sulphate still in the solution. When this has been done the fluid should be evaporated to a small bulk and filtered into absolute alcohol. The peptones are precipitated by this process and may be removed in the same manner as proteose.

Perform the following tests for peptones in solution :

1. Precipitate by tannic acid, phospho-molybdic acid, phospho-tungstic acid, and absolute alcohol.

2. Diffusion. Place some of the solution in a parchment shell and suspend in a beaker of water. After several hours test the water for proteid by the biuret reaction. This test gives a rose-red color with the peptones.

3. Acidulate strongly with acetic acid and then add potassium ferrocyanide. No precipitate.

233. Determination of Proteids Soluble in Alcohol. Certain proteids are not removed from tissues by the saline solution, but after

treatment with this fluid they may be dissolved in dilute alcohol. The principal examples of this class are gliadin and zein.

Material which has been extracted with salt solution, should be washed with water to remove the salt, and then 80 per-cent. alcohol added, which with the water in the material will make about a 75 per-cent. solution. This extraction should be made at a temperature of 40° C. to 50° C. The solvent is drawn off from time to time and fresh alcohol of proper strength added until no more proteid can be obtained. The extracts are filtered and the filtrate evaporated or distilled while the proteid separates from the solution. The precipitate should be purified by treating with absolute alcohol, absolute alcohol and ether, and finally pure ether. The portion rendered insoluble in alcohol by this process may be separated by suspending the proteid in 75 per-cent. alcohol until the soluble part is all dissolved, and filtering. The proteid remaining in the filtrate may be separated by pouring the alcohol into water, and filtering out the precipitated proteid.

234. Proteids Soluble in Dilute Acid and Alkali. After the treatment of material with the solvents as above described, there may still remain a considerable amount of proteid undissolved, which may be removed by the action of dilute acids and alkalies. By the use of comparatively dilute solutions of acids or alkalies the residual proteid becomes albuminate. It is possible to extract the proteids unchanged in some instances however, if it is done in the cold with very dilute alkali.

The material from which other proteids have been removed by methods already described is covered with twice or thrice its volume of 1 per-cent. potassium hydrate. This is allowed to stand for some time at room temperature. The extract is then drawn off and a fresh solution is added. This is repeated at least three times and the extract filtered. The filtrates are neutralized with acetic acid and the resulting precipitate washed with water, alcohol, and ether and, dried to constant weight.

These methods will be found sufficient for the separation of the more important proteids. If other and more detailed methods

are desired, the student is referred to the works of Osborne, Campbell, Wiley and others.[1]

235. The Fats. A fat is a compound of glycerine with fatty acid. Fats are composed simply of carbon, hydrogen and oxygen. Glycerine is a trihydric alcohol and unites with three molecules of a fatty acid to form an oil or a fat. For this reason the neutral fats and oils are usually spoken of as triglycerides. The monatomic alcohols by oxidation give rise to a series of fatty acids. The acid with the general formula $C_{n-1}H_{2n-1}.COOH$ is derived from an alcohol with the formula $C_nH_{2n+1}.HO$. Thus ordinary ethyl alcohol, CH_3CH_2OH, by the removal of two atoms of hydrogen in the process of oxidation gives ethyl aldehyde, CH_3CHO ; further oxidation by the addition of an atom of oxygen forms acetic acid, $CH_3.COOH$. The radicle of acetic acid is called acetyl and when this radicle unites with glycerine, $C_3H_5(HO)_3$, to replace the three atoms of hydroxyl, the result is triacetin, $C_3H_5(O.CH_3CO)_3$, which is a type of a neutral fat. Similarly tristearin, tripalmitin and triolein are obtained from stearic, palmitic and oleic acids respectively. Oleic acid, however, belongs to a somewhat different series, the general formula for which is $C_{n-1}H_{2n-3}.COOH$.

The neutral fats have the following general properties :

(*a*) When pure they are odorless, tasteless and colorless.

(*b*) They are lighter than water.

[1] Wiley, H. W.   Principles and Practice of Agricultural Analysis.   3 :   1897.

Wiley, H. W.   Composition of Maize.   U. S. Dept. of Agric., Div. of Chem. Bull. No. 50.   1898.

Osborne, Thos. B., and Voorhees, Clark L.   Proteids of the wheat kernel, kidney bean and cotton seed.   Conn. Ag. Exp. Sta. 17th Ann. Rep. 175–217.   1893.

Osborne, Thos. B.   Studies of the proteids of rye and barley.   Conn. Ag. Exp. Sta. 18th Ann. Rep. 147–191.   1894.

Osborne, Thos. B. and Campbell, George F.   Conglutin and Vitellin.   Jour. Am. Chem. Soc. **18** : 1–15.   1896.

Osborne, Thos. B.   Some definite compounds of protein bodies.   Jour. Am. Chem. Soc. **21** : 486–493.   1899.

Osborne, Thos. B., and Campbell, George F.   The nucleic acid of the embryo of wheat and its protein compounds.   Jour. Am. Chem. Soc. **22** : 379.   1900.

Cohnheim, O.   Chemie der Eiweisskörper.   1900.

(*c*) They burn with a luminous and smoky flame.

(*d*) They are not volatile.

(*e*) They are readily soluble in chloroform, ether and benzene. They are also soluble in boiling alcohol, from which they separate on cooling, often in crystalline form.

(*f*) They are insoluble in water.

(*g*) With soap they form a fine emulsion.

(*h*) On being decomposed by heat they give rise to irritating acrolein vapors.

(*i*) When boiled with caustic alkali, alkali salts of the fatty acids are formed and glycerine is set free. This process is called saponification.

Fats form a large part of the reserve food substances of plants. They occur in largest quantities as storage material in seeds, many of which, as the seeds of *Ricinus* or *Cocos*, are especially rich in these substances.

The translocation and assimilation of fats is preceded by their division into fatty acids and glycerine. Free fatty acids may or may not be present with fats in a resting condition, but where translocation is going on there is to be found the maximum quantity of free fatty acid. Even at this time very little free glycerine can be detected; it is probably immediately assimilated. Fatty acids, or neutral fats in the presence of fatty acids, readily form an emulsion with sodium phosphate and other reagents and this no doubt facilitates the transformation of the fat.

236. Extraction of Fats. The separation of the fats from the tissues in which they occur is accomplished by grinding up the tissue as finely as possible, and covering it with about twice its volume of a solvent, preferably ethyl or petroleum ether. Petroleum ether is probably best, as it extracts less of other substances. The extraction should be continued until all the fat is removed if the work is of a quantitative nature.

The fat is then separated from the solution by evaporation of the solvent. This should be done over the steam-bath and away from the flame. The odor of petroleum in the fat thus obtained is un-

pleasant and persistent, but may be removed by warming the fat in shallow layers, or better by passing a current of dry CO_2 through the liquid fat in a cylinder. After several hours it should be practically free from the odor of petroleum. Small quantities of water in the extracted fat may be removed by filtering through a dry folded filter paper in a jacketed funnel at high temperature.

237. Qualitative Tests for Fats. On the fat obtained try the following tests :

1. Note its appearance. Olein is fluid at ordinary temperature ; palmitin and stearin are solid. Palmitin crystallizes from ethereal solutions in rosettes of fine needles. Stearin separates from alcoholic solutions on cooling in rectangular or rhombical plates. Olein solidifies at about −50° C. in needle-like crystals.

2. The acrolein test. Decompose a little of the fat by heat in a crucible or other suitable vessel with some potassium bisulphate. Notice the peculiar irritating vapors arising from the decomposition of the glycerine.

3. Shake a little of the melted fat with soap " solution " in a test-tube. An emulsion is formed by the fat separating into minute globules which remain discrete.

4. Determine the melting point. Fill a thin glass spindle with the melted fat, seal the ends and solidify by cooling. Attach the spindle to the bulb of a thermometer and place both in a test-tube half full of water. Clamp the test-tube in a beaker of water suspended in a second beaker of water and heat gradually. Note the temperature at which the fat becomes translucent.

The solidifying temperature of palmitin is about 45° C., and its melting point 50–66° C. Stearin melts at a little higher temperature than palmitin, 55–71° C.

5. Place some of the fat in a casserole with several times its volume of dilute caustic potash solution. Boil for about half an hour. The fat by this time should be saponified. It will now lather with water.

6. Dissolve a little of the soap from the last experiment in a test-tube with alcohol. Add dilute hydrochloric acid and warm until the

fatty acid is liberated and rises to the top in drops. Shake with a few cc. of ether. Draw off the ether and allow a drop of it to evaporate on a glass slide. Examine for crystals of fatty acids.

Stearic acid crystallizes in long rhombical scales or plates. Palmitic acid appears in tufts of fine needles. Oleic acid crystallizes at about 4° C.

7. Test for oleic acid. Evaporate the ethereal solution of the fatty acids. If part of the remainder does not solidify at ordinary temperature it is probably oleic acid. Separate it from the solid acids and add concentrated sulphuric acid and a little cane-sugar. With this treatment oleic acid gives a beautiful red or reddish violet color.

238. The Determination of Organic and Inorganic Matter. Weigh a crucible carefully and place in it the finely divided tissue for determination. Weigh again and then place the crucible in a thermostat to dry at 110° C. When successive weighings show no decrease in the weight of the substance it may be considered dry and a simple calculation will give the weight of the water and its percentage. The dry material in the crucible may now be ignited. For this purpose the crucible should be placed on a wire triangle and the flame of a Bunsen burner applied beneath. Care should be taken that the temperature is not too high. A dull red color at the bottom of the crucible is usually indicative of sufficient heat. All carbon should be carefully burned from the crucible by tilting it on its sides. Carbon which persists in the ash should be worked to the bottom of the crucible with a needle or other small instrument when it will usually oxidize. When burning is complete, cool the crucible and weigh carefully. This weight minus the weight of the crucible gives the amount of inorganic substance in the material.

239. Inorganic Constituents. The quantity and variety of the mineral substances found in any plant depends on the composition of the soil in which it grows. Certain substances however, are essential for the growing plant and among these are calcium, potassium, magnesium and iron. These are not present in the metallic

condition but in combination with acids, forming phosphates, carbonates, sulphates, chlorides, etc. When a plant is burned these substances are found in its ash. Probably they do not all occur in the plant in the same forms in which they are found in the ash, the heat of combustion being doubtless responsible for new combinations. The essential salts of plants may form from 1.5 to 5 per cent. of their dry weight, where these only are to be had, but when much unnecessary salt is available, the percentage of mineral matter is usually much greater.

240. Qualitative Determination of Mineral Constituents. Besides the mineral substances already mentioned as necessary, many others also occur in plants, but for the present purpose it will suffice simply to notice those which are most common.

Ash for analysis may be obtained by carefully burning a quantity of the tissue concerned. The ash should be separated into three parts, first by removing as much as will dissolve in water, then dissolving as much as possible of the remainder in hydrochloric acid, and retaining the residue insoluble in each. Boil the ash with water, filter and wash the residue (Solution I).

Treat portions of this solution as follows :

1. Evaporate a portion to a small quantity and add hydrochloric acid. Effervescence indicates carbonic acid, probably from carbonates of alkali earths. If lead acetate paper is darkened by the escaping gas, sulphur in the form of sulphide is also present.

2. Treat another portion as in 1. Apply a few drops to yellow turmeric paper, and dry at gentle heat. Boric acid is indicated by a red color. Evaporate the solution to dryness and add very dilute hydrochloric acid. Allow to stand for a few minutes and filter. Divide the filtrate into two parts. (*a*) Add ammonium hydrate and magnesia mixture ; phosphoric acid is indicated by a white precipitate. (*b*) Evaporate to dryness on the water-bath with excess of nitric acid. Dissolve the residue in nitric acid and add molybdic solution. A yellow precipitate indicates phosphates.

3. Add silver nitrate till no more precipitate forms. A dark

precipitate = silver sulphide. A white precipitate which does not dissolve in dilute HNO_3 and which darkens in the sunlight = silver chloride.

4. Add hydrochloric acid and heat; render alkaline with ammonia, add $(NH_4)_2C_2O_4$, and allow to stand. A white precipitate occurs if calcium is present.

5. Precipitate calcium as directed in 4, filter, and to the filtrate add NH_4OH and Na_2HPO_4. If magnesium is present a precipitate will be formed, though perhaps slowly.

6. If magnesium is found it must be removed before testing for sodium or potassium. After precipitating the calcium, filtering and evaporating the filtrate to dryness, the residue should be ignited to remove ammonium salts. Heat the residue gently with a little water, add baryta water or milk of lime, free from alkali, till precipitate ceases to form. Boil, filter and add to the filtrate a slight excess of ammonia and ammonium carbonate in mixture. After warming for some time, filter and evaporate the filtrate to dryness with a little NH_4Cl, ignite with low heat until all ammonium salts have been volatilized and dissolve the residue in a little water.

(a) A drop of the concentrated solution held in the flame gives a yellow color if sodium is present.

(b) Add to the remainder of the solution a little hydrochloroplatinic acid, H_2PtCl_6. A yellow crystalline precipitate indicates potassium. It may require some time for this precipitate to form.

The residue from the aqueous extract (Solution I.), after washing, should be treated with hydrochloric acid. Add a few drops of sulphuric acid, and evaporate to dryness. Treat the residue with a little hydrochloric and nitric acid, add water, heat gently and filter (Solution II.). On portions of this solution perform the following tests:

1. Add sodium carbonate with constant stirring until the precipitate formed ceases to redissolve. Add sodium acetate and a little acetic acid. A yellowish white precipitate indicates ferric phosphate.

2. Add ammonium hydrate. A light green precipitate changing to reddish brown indicates iron. If no change in color is observed in a white gelatinous precipitate aluminum is probably present.

3. Add freshly made solution of potassium ferrocyanide. Iron gives a blue precipitate of ferric ferrocyanide.

The residue from solution II, insoluble in HCl, should be washed with water, then fused with excess of sodium carbonate, dissolved in warm water and filtered. Concentrate the solution and while stirring add hydrochloric acid slowly to excess. If silicic acid is present a gelatinous precipitate is formed, which, when evaporated to dryness, yields an insoluble white powder.

241. Enzymes. A number of substances in the plant of unknown composition have the power of producing hydrolytic cleavage in various materials which are used for food, or constructive purposes, and are known as the enzymes, or soluble ferments. They are classified according to the character of the substances upon which they act (See classification of enzymes).

Soluble ferments are prepared from tissues in which they are found by extraction with cold water, dilute glycerine, or dilute saline solution. They are most readily obtained from tissues in which large quantities of reserve food are being quickly digested and translocated, as in the endosperms of germinating seeds. The material for treatment should be finely divided and covered with about twice its volume of the solvent. After standing for twenty-four hours the extract should be drawn off, filtered, and alcohol (95 per-cent.) added to precipitate the enzyme. Other substances such as proteids, will be present in the extract and will be precipitated with the enzymes. The precipitate should be removed by filtering, and dried at low temperatures. The kind of food present in the tissues in which the enzyme was found will suggest the character of the ferment.

242. Determination of Enzymes. The tissue to be extracted should be ground finely and treated with twice its volume of water, glycerine or salt solution for twenty-four hours, and the following tests made :

1. To 10 cc. of 1 per-cent. starch paste add 5 cc. of the extract. A clearing up of the starch mixture occurring after several hours will indicate diastase. Draw off a little of the mixture

from time to time and treat with iodine and with Fehling's solution. The test should be made at 35° C. For control add some of the boiled extract to another portion of the starch mixture. Boiling destroys the enzyme, and this will show whether the changes are due to its action or not.

2. Invertase may be detected by its action in the extract when added to a solution of cane-sugar. In such tests the reducing power of the sugar should be tested first, as it is probable that some reducing sugar will be extracted with the enzyme. After the extract has acted upon the solution for an hour, the reducing power of the mixture should be determined again and the increase accredited to invertase.

3. Cytase, a cellulose-dissolving ferment has been found in fungal mycelia and in a number of monocotyledonous plants. Its effects can be tested upon cotton fiber acidulated with acetic acid. It causes cellulose to swell and gelatinize. Only simple celluloses are affected ; lignified, cutinized, or suberized walls resist its action.

4. Suspend some finely divided coagulated proteid in a few cc. of the extract. Acidify with 0.025 per-cent. hydrochloric acid. The disappearance of the proteid indicates the presence of trypsin.

5. Add to some of the extract an emulsion of the oil from the same kind of seeds from which the extract was made. If lipase is present it will be indicated by the increased acidity of the mixture as indicated by the greater quantity of standard alkali solution necessary to neutralize it after digestion. The increase in the amount of free fatty acid will be due to the action of the lipase. This increase may be demonstrated by adding neutral litmus solution at the beginning of the experiment.[1]

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[1] Directions for more detailed work on ferments may be obtained by consulting the following works :

Green, J. Reynolds. The Soluble Ferments and Fermentation. Cambridge. 1899.

Green, J. Reynolds. Vegetable Physiology. London. 1900.

Effront, J. Les Enzymes et leurs Applications. Paris. 1899.

Osborne, T. B. On the chemical nature of diastase. Conn. Agric. Exp. Sta. 18th Ann. Rep. 192–207, 1894.

X. EXCHANGES AND MOVEMENTS OF FLUIDS

243. Physical Constitution of Protoplasts. Vegetable proto-plasts, except in the lowest forms, are invested by more or less rigid walls composed of a mixture of substances which may be included under the term cellulose. The periphery of the proto-plast forms a plasmatic membrane in contact with the wall, and also a second membrane enclosing the spaces filled with fluid—after the formation of vacuoles—in its interior. Membranes also surround the nucleus and the several kinds of plastids. All of these parts of the protoplast, including the plasma itself, are capable of imbibing water and swelling in different degrees. In regard to solutions of other substances however, these membranes exhibit the most diverse reactions and are by no means permeable to the same substances. The cell walls are permeable to the greatest number, but their capacity decreases when impregnated with waxy and oily substances, as in cork and cuticle. The imbibing power of the plasmatic membranes is regulated by the protoplasm and may be varied from time to time. When two solutions of unequal concentration, or two substances which attract each other, occur on opposite sides of one of these membranes they will diffuse through the separating membrane with a rapidity dependent upon the ease with which they are imbibed by the membrane. If the mem-brane is permeable to one of the substances alone it will pass through alone, and the other will remain stationary.

244. Imbibition. The imbibition of a fluid by a solid is due to the energy of surface tension, or attraction, existing between the particles of the two substances. This causes the fluid to pene-trate between the particles of the solid and separate them as far as their cohesion will allow. The beginning of the process is ac-companied by a display of enormous energy which decreases as the expansion of the solid proceeds.
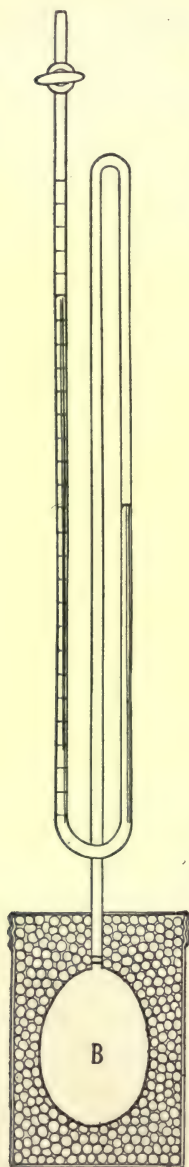
FIG. 84.

245. Increase in Walls by Imbibition. Cut longitudinal and transverse sections from stalks of *Laminaria* which have been preserved dry, or in alcohol, and mount in alcohol. Measure the thickness of the walls by means of a micrometer eyepiece. Measure changes in length of a strip 2 cm. long. Now put a strip of blotting paper in contact with the edge of the cover-glass on one side and run a large drop of water in at the other as the alcohol is withdrawn. Compare the increase in the thicknesses of the wall in the three axes of the stalk.

246. Energy of Imbibition. Secure a screw-topped jar, at least 6 cm. in diameter and 10 cm. in width, or use a Mason fruit jar. Make a manometer by sealing one end of a glass tube with an internal diameter of 2 mm., and then bending it twice at right angles to form a U the arms of which are at least 15 cm. long, with the free open arm twice this length. Thrust the free arm through a hole of sufficient size cut through the metal cover of the jar. Run in enough mercury so that it will stand at about 8 cm. in both arms at normal pressure and run water into the free arm by means of a minute glass or metal tube until it is full. Care must be taken to have it rising to the same height in the arms. Now fill a rubber bulb of a capacity of about 100 cc. with water and fasten to the open end of the manometer, and after it is in place run a wire along the tube into the bulb to relieve any compression set up. Withdraw the wire and use it to bind the mouth of the bulb tightly to the manometer arm. The wire

should be wound once around the rim of the bulb and the ends twisted with a pair of pincers. Hold the bulb in the center of the jar and pour seeds of pea, bean, or soja bean, around it until the jar is completely filled. The seeds should be compacted by shaking from time to time. Now bring the top to its place and screw on. Set the jar in a larger vessel with the manometer arm extending above and outside it. Pour water in the vessel in such manner that it will completely cover the jar containing the seeds. Mark the exact height of the mercury in both columns, and adjust the outer arm to a perpendicular position. Measure accurately the length of the column of air above the mercury in the enclosed column. As the seeds in the jar swell, the bulb will be compressed and the mercury driven up in the outer arm of the manometer. Note the length of time elapsing before imbibition begins, as denoted by the rise of the mercury column, and measure the length of the column of air at intervals for a day. Part of the effect will be due to the osmotic power of substances in the living cells. The actual amount of pressure is to be calculated by Boyle's law. The volume of a gas varies inversely with its pressure. Thus if the column of air originally measured 8 cm. and is compressed to 6 cm. in length the pressure will be eight-sixths of an atmosphere [1] (Fig. 84).

An iron cylinder was filled with peas and fitted as in Fig. 84, on February 23, 1901, and the following observations made :

Time.	Length of Column of Air.
9:30 A. M.	6.30 cm.
10:00 "	5.90 "
10:06 "	5.50 "
10:10 "	5.20 "
10:35 "	4.60 "
10:42 "	4.30 "
10:51 "	4.10 "
11:11 "	3.80 "
11:40 "	3.40 "
11:50 "	3.20 "

[1] Coupin, H. Recherches sur l'absorption et le rejet de l'eau, par les graines. Ann. Sc. Nat. Bot. 8. 2 : 128. 1895.

13

Time.	Length of Column of Air.
12:50 P. M.	1.60 "
2:25 "	2.00 "
3:30 "	1.70 "
7:20 A. M.	1.20 "
9:00 "	.80 "
4:ᴄ0 P. M.	.78 "

The total duration of the experiment was 30 hours, and the final pressure attained was sufficient to compress a column of air from a length of 6.30 cm. to .78 cm. and the amount is indicated as $630/78 = 8$ atmospheres. This pressure was maintained for two days and then began to decrease slowly, showing but 1.2 atmospheres a week later.[1]

247. Movements Caused by Imbibition. Secure a few awns of *Stipa avenacea*, which are usually curved at right angles midway.
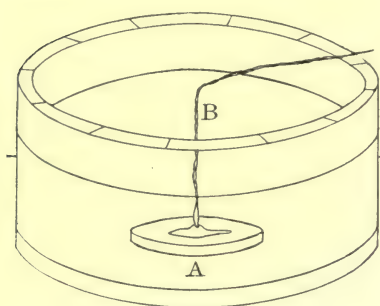
FIG. 85. *B*, awn of *Stipa avenacea* fastened to metal disk *A*, and set in dish of water to give hygroscopic movements.

Warm a cent piece and put a drop of sealing wax in the middle of one side. Thrust the basal end of the awn into the wax, and as soon as it is cool set it in the center of a glass dish about 2 cm. in diameter, and about 3 cm. deep. Mark the mouth of the dish into fractions of a circle. Now fill the dish with water and note the movements of the bent awn. The terminal portion will sweep around the dish like the hands of a watch. After a half hour pour the water from the dish and set in a warm place and follow the reverse movement. The awn will not return to the point from which it started for some time, perhaps days, since the last of the water taken up is lost very slowly. The movements are the result of forces set up by the imbibition of water

[1] MacDougal. Force exerted by swelling seeds. Jour. N. Y. Bot. Garden. **2** : 39. **1901.**

in the excentric spirally arranged walls of sclerenchymatous cells
with narrow lumina (Fig. 86).[1]

248. Osmose in Cells. The cell
is an osmotic system of mem-
branes, each with its own specific
permeability; in addition the
outer and most permeable of
these, the wall, is rigid and pos-
sesses great structural strength.
When the plasmatic membranes
become filled with solutions
and press against the wall, it is
stretched only slightly and as-
sumes a state of great rigidity,
and the cell in such condition of
distention is said to be in a state
of *turgidity*. If a turgid cell is
immersed in a solution to which
the outer wall is permeable, and
which has a higher isotonic
coefficient than the solution held
in the plasmatic membrane,
water will be withdrawn from the
plasma, and it will shrink away

FIG. 86. 1, cross section of half of
the axis of an awn of *Stipa Avicenacea*,
showing disposition of the mechanical
cells. 2, portion of a single cell seen in
longitudinal section, in an air-dry condi-
tion, showing spiral arrangement of the
wall. 3, optical section of a portion of
2 taken from *x* in 1. 4, portion of sim-
ilar cell after treatment with macerating
fluid. 5, diagram showing the resultant
of forces that may give rise to torsional
movements. × about 75. After Mur-
bach.

from the wall, and is said to be plasmolyzed. On the other hand,
if organisms accustomed to living in concentrated solutions are
placed in pure water, so much of this substance may be taken
up that a pressure sufficient to rend the wall may be generated.
This may be seen in some pollen grains, and Pfeffer mentions
that *Aspergillus*, under such circumstances, sets up a pressure of
160 atmospheres (see appendix).

[1] Murbach, L   Note on the mechanics of the seed-burying awns of the *Stipa
Avicenacea*.   Bot. Gazette. **30** : 113.   1900.

Remer, W.   Beiträge zur Anatomie und Mechanik tordirenden Grannen bei
Gramineen, nebst Beobachtungen ueber den biologischer Werthe derselben.   Bres-
lau.   1900.

249. Plasmolysis. Mount a filament of *Spirogyra*, hair from *Cucurbita, Tradescantia, Lycopersicum*, or a portion of the epidermis of a young plant in distilled water. Note the condition of the total inclusion of the cell, and movement if present. Place a drop of 5 per-cent. solution of potassium nitrate at one side of the cover-glass, and draw out the water from the other side with a strip of blotting paper. Note effect on cell. Draw and replace the preparation in distilled water as before, noting result.

250. Permeability of Plasmatic Membranes to Coloring Matter. Fill a large culture dish with a solution of water and methyl-blue, in the proportion of one to a hundred thousand, and in it place filaments of *Spirogyra*, and sprigs of *Philotria*, allowing them to remain 24 to 48 hours. Examine with magnification of 400 to 500 and note the presence of coloring matter in the vacuoles, showing that the methyl-blue has passed the membranes of the cytoplasm without the latter being stained. The accumulation of the dye in the vacuoles is due to the fact that it is converted here into a form to which the plasma is not permeable. It forms precipitates in *Spirogyra*, but remains fluid in *Philotria*.[1]

251. Osmose ; Change in Osmotic Qualities of Membrane Affecting Permeability. Soak a section of parchment tubing 25 cm. long and 5 cm. in diameter, in water for an hour, than pleat a small section at one end, double back and tie firmly with a wrapping of cord. If parchment capsules are procurable this will be unnecessary. Fit a perforated cork or rubber stopper to the other end and secure it firmly with many wrappings of cord. Next provide a separatory funnel fused to the horizontal arm of a T tube. Insert one free arm of the T tube in the perforation in the stopper and to the other end fit a capillary tube by means of a section of rubber tubing bound with wire. The capillary tube may be 2 m. in length and should be held upright by suitable supports. Fill the parchment tube with a saturated solution of cane sugar, then close the stopcock of the funnel. Immerse the

[1] For full discussion of the entire subject see Pfeffer's Plant Physiology, **1** : 70–174. 1900. Also see appendix.

parchment in a cylinder filled with water and note the rise of
the column in the capillary tube during the next few hours, due
to the attraction of sugar for water. Wash
the membrane thoroughly and fill the capsule
with a solution of di-sodic phosphate colored
deeply with methylene-blue. Note the rise
of the column and also that some of the color
is diffused out into the water of the cylinder.
Refill again with di-sodic phosphate and me-
thyl-blue and immerse the capsule in a solu-
tion of calcium nitrate (1 per-cent.) for a
short time. Now place in a cylinder of water
as before. The calcium nitrate forms a pre-
cipitate in the membrane, and this changes
its permeability so that it does not allow the
coloring matter to pass through it to any
great extent. Similar changes take place in
walls and membranes with analogous re-
sults.

252. Turgidity. If the outlet tube of the
osmometer in the last experiment is closed,
the parchment cylinder is expanded to its
fullest capacity for stretching and becomes
hard and rigid, resembling the condition of a
cell under similar circumstances. The princi-
pal substances in protoplasts which attract
water from the outside are sugars, salts of
organic acids and potassium nitrate. The
plasmatic membranes show specific resistance
to the diffusion of these substances outwardly,
or else the cell would quickly lose its power of inducing
endosmosis and maintaining turgidity. The substances ab-
sorbed in this manner are quickly changed into some form not
so readily diffusible, so that a turgid cell has a constant stream of
solution passing through its walls and plasma into the vacuoles.
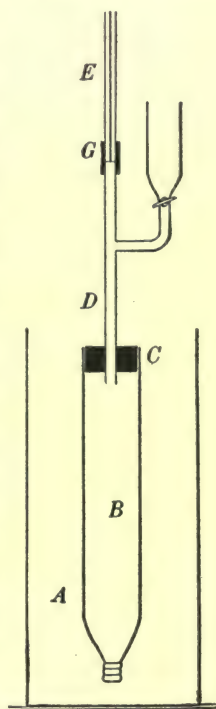
FIG. 87. Osmometer.
A, glass vessel contain-
ing distilled water. *B*,
parchment cylinder. *C*,
stopper. *D*, T-tube. *E*,
capillary extension tube.
G, joint made with rub-
ber tubing and wired.

The turgidity of the active cells is the chief factor in producing the rigidity of the soft-bodied plants.

253. Estimation of the Force of Turgidity in Tissues.   Take an actively growing flower stalk as those of the cowslip, honeysuckle, plantain, or growing petioles of some acaulescent *Oxalis*, and place in a concentrated solution of cane sugar for four hours. The stalk should be about 100 g. in length.   Before placing in the solution, make a thin India ink mark near each end, and measure the distance between the marks accurately with a milli-meter scale.   Take the stalk from the solution and note its limp condition.   Lay on a piece of glass plate and measure the distance between the marks and determine the amount of shrinkage caused by the plasmolysis of the tissues caused by the cane sugar.   Now lay on a board slightly wider than the length of the stalk, and clamp the thin end of the stalk to the board by means of a screw and a piece of wood or cork, leaving the ink mark in sight.   Tie a thin cord to the other end and pass it over a small pulley set in the edge of the board.   Fix a small scale pan to the cord and load weights into it until the distance between the ink marks is restored to the original measurement.   The amount of the weights used represents the force exerted by the turgidity of the stem.   Measure the diameter of the stalk, and compute area of cross section.   Calculate the turgidity in atmospheric pressures. A pressure of one atmosphere is equal to 10.3 grams per sq. milli-meter.   The weight of the scale pan should be taken into account. The stalk may be suspended vertically and the pan hung to its lower end when the weight of the pan and a fourth of the weight of the stalk are to be added to the amount, which will still leave a slight error.

Fig. 88.   Apparatus for determining amount of tur-gidity. *A*, wooden clamp. *B*, board with pulley at *R*. *G*, scale pan with weight. After Detmer.

254. Tensions of the Tissues. The wood and other dead tissues in a stem are not turgescent and hence are in a passive condition. The pith and cortex are usually not equally turgescent, so that a number of tensions or strains are set up in stems where these tissues are bound together. If the stem is separated into its different tissues they will lengthen or shorten accordingly as they have been compressed or stretched in the plant. These tensions are also exerted in radial and tangential directions.

255. Longitudinal Tensions. Secure young and rapidly growing tips of stems of *Sambucus, Nicotiana,* or *Helianthus,* or flower scapes of *Taraxacum.* Split into quarters and note the positions assumed. Separate the pith, wood and cortex of a portion of a stem of *Helianthus* 50 cm. long with a sharp knife. Measure the length of the stem accurately before doing so, and then find exact length of the pith, wood and cortex, a few minutes later. The pith may extend to 54 or even 56 cm., while the wood and cortex will change but slightly.

256. Absorption of Liquids. Every living cell exerts its own osmotic effect upon fluids with which it comes into contact. These fluids may permeate the wall only, and it is possible for a substance to traverse the entire length and thickness of the body of a plant in this manner, or they may penetrate the plasmatic and vascular membranes. The living cell differs in its action from the osmometer in two particulars : First, the substances attracted into the cell may be converted into solid or non-diffusible form leaving the original attractive compounds to draw in a new supply of the same substances, continually ; secondly the plasmatic membrane is controlled in such manner that it allows the diffusion of some substances and bars others, in a manner not explainable by purely physical laws ; the relations of the plasmatic membrane to any given substance may change from time to time also. The so-called selective power of plants rests upon these facts. By this faculty different cells and different species take from the substratum entirely different substances.

257. Absorbing Organs.   The lower forms absorb liquids over their entire surfaces, but the higher have differentiated the roots as special fixing and absorbing organs.   The outer cells of the roots are generally extended in the form of long tube-like extensions which increase the surface capable of carrying on osmotic absorption many fold.   The root hairs penetrate between the finer particles of the soil, coming into direct contact with the thin layer of hygroscopic water surrounding each particle.   The hygroscopic water contains some of the mineral salts of the soil in solution, and a selection of these with the water is drawn into the hair by the osmotic attraction of the protoplasm and solutions in the vacuoles.   The concentration, or proportions of the molecules of the salts and water are also determined by the cells, irrespective of the strength of the solution in which they may be found.
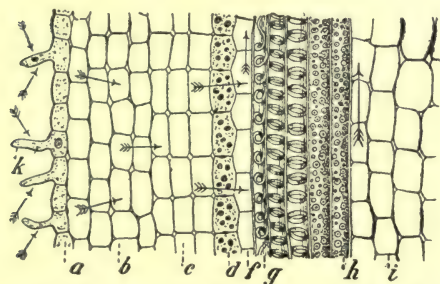
Fig. 89. Diagram of radial longitudinal section of young root. *a*, epidermal layer with root hairs. *b*, external cortex. *c*, internal cortex. *d*, endodermis. *f*, pericycle. *g-h*, vessels. *i*, pith.   Fluids absorbed move in directions shown by arrows.   After Belzung.

The absorption of water from the soil continues until the osmotic attraction of the substances in the root-hairs is equal to the surface tension of the thin layer of water coating the soil particles.   If this is not renewed, there will come a stage in the procedure when the plant will not be able to obtain a further supply from the soil, although it contains an appreciable quantity which may be driven off by heat amounting from from 1.5 to 8 per cent. of its total weight in different soils.

258. Structure of an Absorbing Root.   Cut a thin cross-section of a monocotyledonous root of any convenient plant and note the form of the root-hairs, the cortex, endodermis if present, and the central cylinder.   Ascertain if passage cells are present in endodermis.

259. Bleeding Pressure, and Nectarial Excretion. The continued absorption of solutions by the long root-hairs sets up a great osmotic pressure interior to the plasmatic membranes, and the exudation pressure in these cells forces some of the fluid out of the hair into the cortex at the base. Similar exudation pressure in the cortex forces water into the vessels and tracheids, and this process continues until the column of water thus formed mounts up in the stem and reaches the apices of low, rapidly growing species. Action of this character is liable to occur in all parts of the plant where turgescent active tissues are in contact with xylem, and is not peculiar to roots. Thus an excised stem placed in water may show a bleeding pressure. The exudation of water from the tips of laminae and from some water pores is due to the same series of causes. The excretion of nectar from glands, stigmatic surfaces, and certain water pores is due to the osmotic attraction of sugars formed external to the walls of the cells lining the gland.[1]

The exudation from a plant diminishes as the turgidity lessens, and also with low temperatures, while the absence of oxygen and the influence of anaesthetics inhibits the excretion, though the cells may be turgid. Exudation pressure shows a seasonal and a daily periodicity, in plants in the temperate zone. It is usually greatest in the early spring before the beginning of growth, and least during the period of greatest transpiration. The amount of water excreted by a plant in this manner may be greater than its own bulk, and it may be thrown out with a pressure exceeding one atmosphere in certain instances, though usually much below this.

260. Measurement of Tension of Fluids in Body of Plant. The tension of liquids in cells may be best estimated by an analysis of the turgidity by means of plasmolysing agents of known isotonic value (See Appendix). The tension under which liquids and gases are found in the non-living elements, and in the intercellular

[1] Trelease, W. Nectar: its nature, occurrence, and uses. 1879.

spaces may be measured however, only by connecting the body of
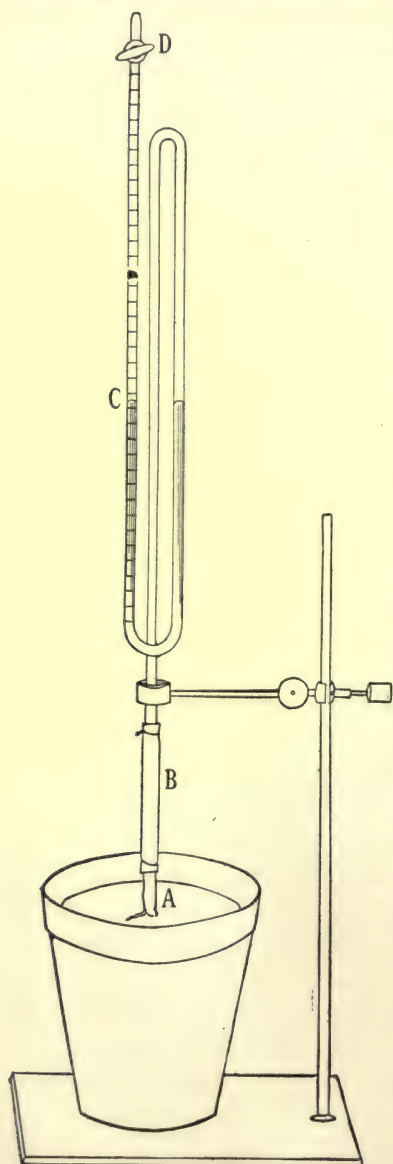
the plant with some form of a manometer by which this pressure is transmitted to the instrument directly. The friction encountered by fluids in their expansion and contraction in the narrow spaces in the plant, and the difficulty of making perfect fittings with the apparatus are such, that all results are only approximate, and generally do not indicate the full extent of the compression or expansion under which the free fluids of the body are sometimes found. Very rarely does the tension of the gases and the liquids in the trachea and intercellular spaces coincide with that of the external atmosphere. Tests of this character are necessarily confined to plants with firm woody stems and branches, and may be made as follows : Provide a manometer of the closed arm type, which may be made by sealing one end of a glass tube and then bending it into the form of a U, the arms of which are at

FIG. 90. Measurement of tensions of fluids in stems. *A*, stump of stem. *B*, section of rubber tubing. *C*, level of mercury in manometer. *D*, stopcock.

least 15 cm. long. The free end of the open arm should be bent in the same plane at right angles for convenience of attachment. The most convenient form is furnished with a stopcock in the closed arm, as in Fig. 90, but in the use of this instrument great care must be taken to have the fitting perfectly air-tight under possible pressures of several atmospheres. Fill the manometer to half the length of both arms with clean mercury, with air at atmospheric tension in the closed arm. Fill the open arm with distilled water by the aid of a minute metal or glass tube. Branches and stems ranging in diameter from that of the manometer tubing to several cm. in thickness may be tested by the use of adapters. In testing the tensions in stems of the approximate diameter of the manometer arm, cut off the stem cleanly with a sharp knife and bind to the stump a section of heavy rubber tubing 6 cm. in length. Quickly fill with water and place a short section of fine wire in the tubing. Now lift the manometer and drive the open end down into the rubber tubing taking care to admit as little air as possible, although a few bubbles will not noticeably vitiate the results. The wire, which now lies between the rubber tubing and the manometer tubing, and which served to allow the escape of superfluous water, may be withdrawn, and the rubber tubing bound firmly to the manometer by means of wire clamped and twisted by means of a pair of pliers. Note the height of the mercury column in both arms of the manometer, and measure the exact distance from the mercury to the end of the closed arm. Measure this distance three or four times daily for a week. If exudation pressure, ordinarily known as "root-pressure," is present the air in the closed end of the manometer arm will be compressed. In accordance with Boyle's law the volume of air in this arm varies inversely with the pressure. Thus if the column of air in the closed arm measured 8 cm. in length at the beginning of the experiment, and at the next observation it was found to be 6 cm., the pressure indicated is 8/6 atmospheres. Or if the column of air measures 10 cm. on the second observation, the pressure will be 8/10 atmosphere, and a

partial vacuum is indicated. Positive pressures of more than an atmosphere have been measured, and negative pressures in which the gases in the plant exhibited but half of the barometric pressure have been recorded. Interesting results may be attained by the attachment of several manometers to branches of a young tree at various heights, when it may be seen that positive pressure has but little connection with root action.

261. Guttation, and Action of Nectaries. Grow a number of seedlings of *Zea* in a pot, and when the blades are a few centimeters in height, cover with a bell-jar, and note the gathering of drops of water on the tips and margins of the leaves. Note similar appearance of drops of water on margins of leaves of any plant covered with a bell-jar for a few hours. Examine the structure of the nectaries of *Passiflora*, *Cassia*, or of any convenient plant and note the structure of the cells lining the nectarial cavity; under what conditions is nectar excreted ? [1]

262. Relations of Plants to Gases. Plants are especially concerned with oxygen, nitrogen and carbon dioxide, and the diffusion of these substances through membranes is governed by the same general laws of osmose, as the passage of liquids. Gases however, penetrate entire membranes only when dissolved in the liquids, with which the membranes are permeated. Carbon dioxide diffuses the most readily, and nitrogen the least readily. Membranes impregnated with wax and other substances, as in cuticle and cork, contain but little water of imbibition, and hence the diffusion of gases through such membranes is very slow. The outer covering of the shoots of plants is converted into cuticle or cork in a great majority of instances for protection and conservation of the body of the plant, and this covering is furnished with a large number of openings through the epidermal layers by which the external layer is connected with the spaces between the cells of cortical tissues. Two special forms of such openings

[1] Wieler. Das Bluten der Pflanzen. Cohn's Beit. z. Biol. d. Pflanzen. **6** : 1. 1893.

may be mentioned : lenticels which furnish connection between the cortex of stems and roots, and the outer air through the cork, and stomata connecting green tissues of leaves and other organs with the atmosphere. The vessels and tracheids which usually contain gases in mature plants, have no direct connection with the air in the intercellular spaces. Any exchange with them must take place by osmose through one or more membranes, since it is impossible to force gas through a membrane by pressure as a liquid might be transmitted. The withdrawal of water from cells by drying out, or diffusion, may reduce the pressure below that of the atmosphere, and in some instance results in an almost perfect vacuum, since the air may not penetrate the wall except by diffusion, unless actual openings are present. Then again it is to be said that carbon dioxide diffuses much more rapidly than oxygen, so that cells in which rapid respiration is in process show a reduced pressure. It is to these causes that the negative pressure of the shoots and branches of large woody plants is principally due. The unequal diffusibility of the atmospheric gases also varies the composition of the air enclosed in the closed vessels of a plant.

The gases in the intercellular spaces may diffuse with great rapidity into the thin-walled cells with which they are in contact. The gaseous exchange between the plant and the atmosphere is regulated to some extent however, by variations in the width of the epidermal openings, i. e., the stomata and lenticels. The stomata are in general under the control of mechanisms by which they may be opened or closed in a few seconds, while the lenticels undergo seasonal changes. The diffusion of the gases of the air through the stomata and lenticels is not exactly similar to the rate through capillary openings. Thus it has been found that the flow of gas through a tube is proportional to the sectional area of the column of gas. It is known however, that if the flow is partially obstructed at any point by a thin septum pierced with a circular aperture, the rate of flow across unit area of aperture is greater than it would be across an equal area

of the unobstructed cross section.[1]   This condition is imitated by the arrangement of stomata in leaves, which are not to be considered as simple capillaries in studies upon gaseous interchange. The rate of gaseous interchange between leaves and the air, based upon simple measurements of the stomata will thus be found faulty.

263. Diffusion of Gases through Coating of Fruits.   Smooth both ends of a glass tube 60 cm. long and with a bore 5 mm. in diameter.   Fit to one end a bored cork in such manner that the top of the cork and tube shall be flush.   Work a surface of soft sealing wax over the cork and edges of the tube, then cut a circular piece of fine wire gauze and cover over the cork and tube, imbedding the gauze in the warm wax.   Cut a circular piece of the rind of a squash or pumpkin, trimming away the inner layers until it is not more than 2 mm. thick.   Lay on top of the gauze and seal around edges with wax, being careful not to burn the material.   Place the tube nearly horizontal with the closed end lowest and fill completely full with distilled water, being careful not to displace the fittings of the closed end by cracking the brittle wax.   Now place the finger over the open end and stand upright in a dish of mercury, allowing no air to gain entrance. Displace the mercury in the tube with carbon dioxide, or oxygen, until it is at the same level in the tube and dish.   Measure the height of the column of mercury as it rises in the tube, by readings, daily, until it sinks to its former level.   Make coincident readings of the barometer.   Compare the structure and condition of the plant material at the beginning and end of the experiment, or compare fresh and treated portions.   It will be found that carbon dioxide diffuses rapidly through the moist plant material, for four or five days very rapidly, until the column of mercury reaches a height of 9 to 12 cm. and then that the drying out of the material makes cracks or openings through which the atmospheric

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[1] Brown, H. T., and Escombe, F.   Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants.   Annals of Botany, **14**: 537. 1900.

gases are drawn in by filtration pressure.   Repeat the experiment, and suspend a small dish of water near the top of the tube, from which a strip of filter paper runs to the membrane and keeps it moist.   In an experiment by the author a grape skin permitted
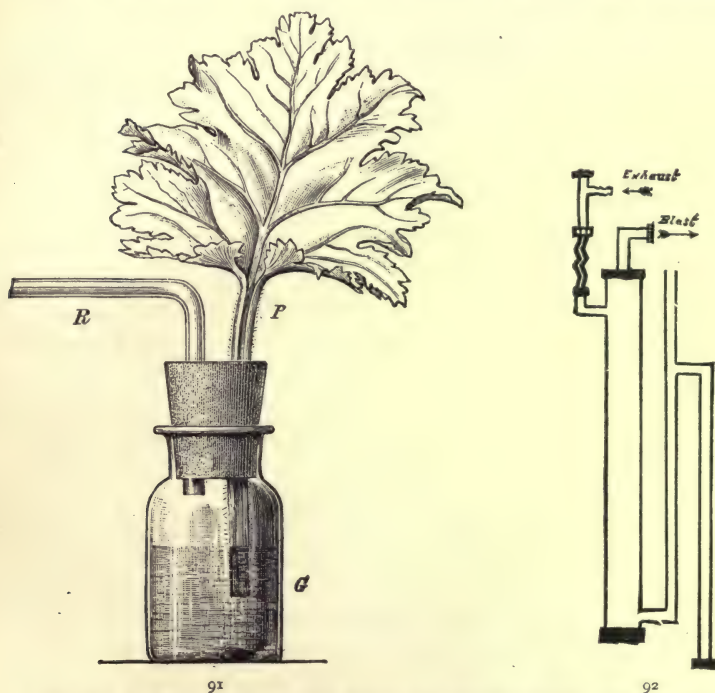
FIG. 91.   Demonstration of the passage of air through stomata, spongy parenchyma of leaf, and cortex of petiole.   *G*, small bottle containing distilled water in which is immersed the lower end of a freshly cut petiole of *Primula Sinensis*.   *R*, tube through which the air in the bottle is exhausted.   After Detmer.

FIG. 92.   Diagram of force and blast pump, to be used in drawing or forcing air through leaf.

the diffusion of carbon dioxide to lift a column of mercury 26 cm. in height, which was maintained 14 months.

264. Diffusion Through a Waxy Membrane. Repeat experiment as above, using the thick waxy skin of a " winter " apple, or a skin of a grape.   Note the steady rise of the column through

a long period and its ultimate maintenance at the maximum elevation. Such membranes contain but little water of imbibition although sufficient to allow diffusion. This amount is not lost by desiccation, so the membrane continues to act osmotically and does not become cracked, or permeable to gases under filtration pressure.

265. Diffusion of Gases Through Leaves. Repeat experiment as above, using leaves of *Ficus*, oak, or any convenient plant with a firm leaf. The section of the leaf should be fastened to the apparatus with the edges sealed to prevent the passage of gas through the intercellular spaces into the air.

FIG. 93. Portion of branch of oak showing lenticels. After Bonnier and Leclerc du Sablon.

266. Connection of Air in Cortex and Spongy Parenchyma of Leaves with the Atmosphere Through the Stomata. Fit a leaf of *Primula Sinensis*, or *Prunus*, to a stopper by boring a suitable hole through the stopper and passing the petiole through it, then sealing with gelatine or wax. Bore a second hole through the stopper and insert a short section of tubing bent at right angles. Force the stopper into a bottle of proper size half full of water. Connect tube with filter pump or suck with the mouth. Note the streams of bubbles pouring from the end of the stem. Continue until it is demonstrated that the bubbles are produced by air passing through the leaf and petiole, not by expansion of the gases in the intercellular spaces. If the leaf is submerged, and air forced into the petiole by blowing with the mouth, or force pump, bubbles may be seen to arise from the surface from the air coming through the stomata. The openings of the stomata soon become filled with water, after which the passage of air may not be demonstrated (Fig. 91).

267. Connection of Air in Cortex of Branches with Atmosphere through Lenticels. Examine a branch of *Salix*, *Sambucus*, *Syringa*, or *Populus*, and a number of small, rough areas slightly

raised above the surface of the bark will be noticed.   These are
lenticels.   Cut cross sections of bark and note structure.[1]

Cut a section of a twig bearing lenticels and pass it through a
stopper holding a piece of bent glass tubing, as in the previous
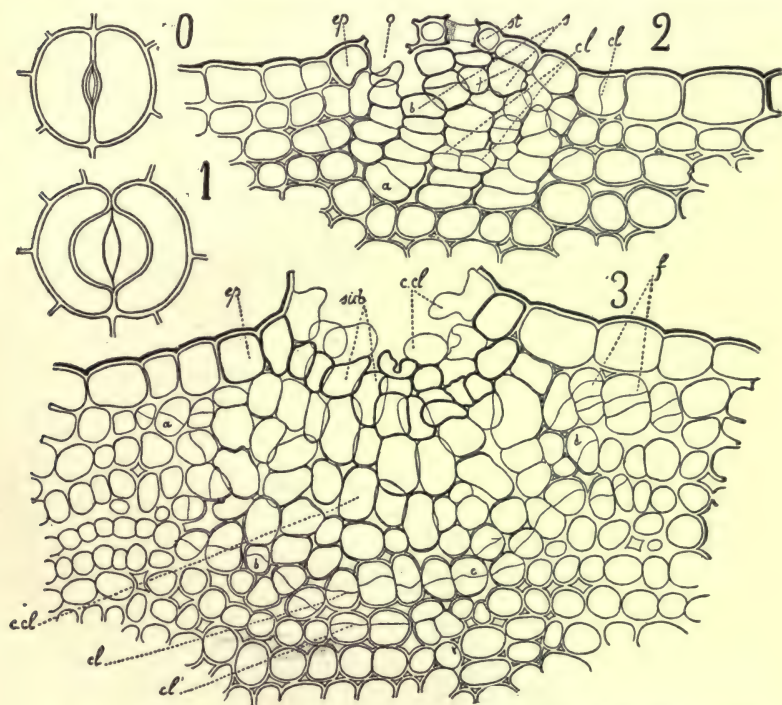
FIG. 94.   *O*, stoma on stem of *Sambucus nigra* from which a lenticel is formed.
1, stoma in the first stage of development toward the formation of a lentical.   **2**,
young lenticel formed by rupture of epidermis near the stoma.   *ep*, epidermis, suberized.
cl, cells of cortex and epidermis undergoing division.   *a*, *b*, cells derived from an
initial cortical cell by repeated division.   **3**, *c*, *cl*, atrophied cells.   *sub*, cells with sub-
erized walls.   *a*, *b*, *c*, *d*, concave layer of active cells.   *cl*, *cl*, cortical cells beginning
division.   After Devaux.

experiment.   Seal in the stopper without abrading the bark, and
also seal the upper end of the twig carefully with wax.   Now
apply suction to the glass tube, and note the exit of air-bubbles

[1] Devaux, M. H.   Recherches sur les lenticelles.   Ann. Sc. Nat. Bot., 8.
12 : 1.   1900.

from the lower end of the twig.   If the lower end of the twig is
also sealed and the suction applied before water has had time
to penetrate the lenticels immersed, the air may be forced out
through the lenticels on the lower end of the twig.   The bottle
should be completely full of water in this test.   If a short sec-
tion of a branch is sealed at both ends, then laid in a dish
of water and warmed slowly, the heated and expanding air may
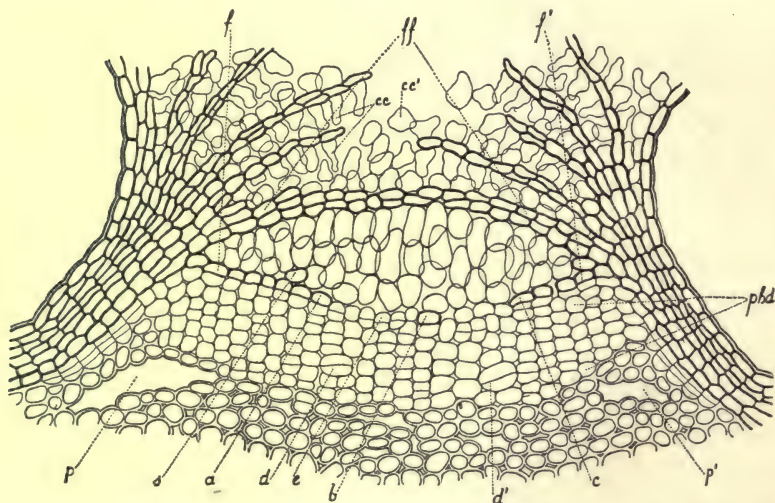
FIG. 95. Lenticel from branch of *Coriaria myrtifolia*, one year old: *ff*, restraining
layers, complete and unbroken. *f'f'*, beginnings of formation of restraining layer. *phd*,
phelloderm.   After Devaux.

be seen escaping from the lenticels.   Seasonal variations in the
readiness with which lenticels conduct air are to be found.   In
some species they are nearly closed in winter.

268. The Length of Free Passages in Vessels.   The vessels of a
plant show extremely long sections of lumina free from septae,
and by lateral connections furnish a free air passage for long dis-
tances in stems.   The length of such segments may be demon-
strated as follows : prepare sections of branches of *Salix*, *Populus*,
or *Syringa* 20 to 50 cm. long, and lay in a trough of water.

Now cut a few centimeters from each end and fasten the end of the branch originally uppermost to a glass tube 6 cm. in length by means of a section of pressure tubing (rubber). Now connect the glass tube with an air pump, or filter pump, and dip the lower end of the twig in a solution of ferric oxychloride. This may be made by adding 3 parts of water to 1 of the officinal preparation of *liquid ferri oxychlorati*, which may be procured of pharmacists. Exhaust the air from the upper end of the branch. If the fluid exuding from the branch, a quarter of an hour later, is colorless, cut away a few centimeters from the lower end of the branch and immerse the newly cut surface in the liquid. Repeat at same interval until the brown fluid appears at the upper end of the branch. The length of the branch when this occurs gives the maximum extent of free air communication in the vessels. The liquor is colloidal and may not be forced through membranes. If the first test has been performed with a twig two years old, repeat with one of the same species five or six years old, and note the increased length of the free air communication. The results will be approximate.
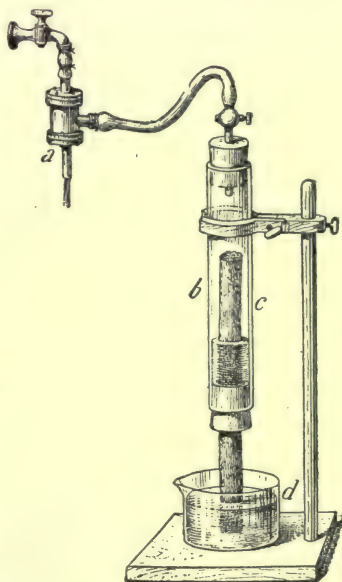
FIG. 96. Demonstration of the length of air-passages in woody stems. *a*, filter pump attached to water tap. *b*, large glass tube in the lower end of which is fixed a branch of chestnut, standing in a dish containing coloring fluid, or injection material. After Belzung.

269. Permeability of Wood to Air. Make some rods of wood from the outer portion of newly-felled trees of *Abies*, or *Taxus*, which are entirely free from dry rot, and insert the short arm of one end in a U-tube and seal tightly with wax. The long arm

of the tube should be at least 80 cm. in length.   Set the prepara-
tion in a tall jar filled with water, and pour mercury into the long
arm of the tube.   After the mercury reaches a certain height the
air between it and the wood is forced through the wood very
slowly and may be seen to bubble up in the water.   This is due
to the presence of very small intercellular spaces.   The amount
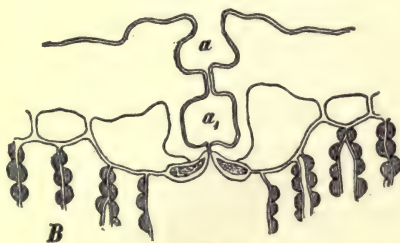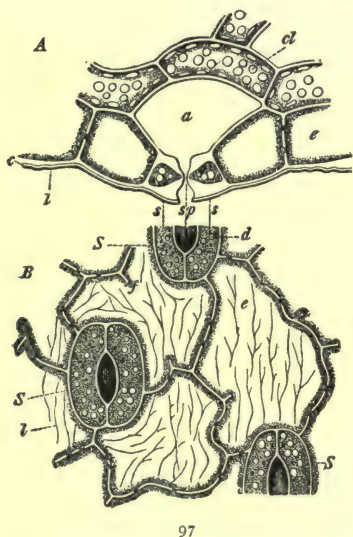
FIG. 97.   Epidermis and stomata of the lower surface of leaf of *Helleborus foetidus;*
A, in cross section ; *B,* surface view.   *e,* epidermal cells.   *c,* cuticle.   *l,* strengthen-
ing ridges of the outer walls.   *f,* folds of the lateral walls.   *S,* stoma.   *s,* guard
cells.   *sp,* opening.   *a,* stomatal chamber.   *cl,* mesophyl.   *d,* chloroplasts.   After
Prantl.

FIG. 98.   *A,* cross section of stoma of *Cypripedium venustum,* with large entrance
chamber shown at *v.*   *B,* cross section of stoma of *Dasylirion filiferum* with entrance
chamber divided into two parts, by folds in the walls of the bounding epidermal cells.
After Haberlandt.

of air passing in this manner is small.   The tracheids are imper-
vious to gases under pressure when wet.

270. Stomata.   The elements of the epidermis join closely to-
gether, so that no facility is afforded for gaseous interchange with
the atmosphere, under the influence of filtration pressure.   At
numerous points on the surface of the leaf regular pores are

formed by the splitting of epidermal cells, the halves of which become guard cells with the power of movement in such manner as to open or close the pores. The origin of the transpiratory openings among the lower forms may not be explained in this manner in all instances, however. The action of the guard cells may be best shown by Fig. 99. These cells are attached to the epidermal cells in such manner that the lower side away from the surface of the leaf is free, and the wall nearest the pore is comparatively thin, while the surface wall, and the one parallel to it, is very much thickened. Any increase of the turgidity of the guard cell tends to force the surface and inner walls farther apart, and to diminish the convexity of the thin wall extending toward the center of the opening. Decrease in turgidity causes the reverse action, and the stoma is closed. This action is somewhat modified by the behavior of the neighboring epidermal cells, which play a very important part in controlling the transpiratory openings in some species. The transpiratory openings of some of the lower forms are permanently open and may not be controlled. Wilting of the leaf, compression of the stem, dry atmosphere, electric stimulation (strong), darkness, and strong mechanical stimulation cause the closing of the pores of the stomata, while light and heat as well as prolonged darkness cause them to open.[1] It is to be said that the stomata do not close their pores so tightly that some gaseous diffusion may not take place through the diminished opening. Stomata are generally found on the lower (outer) surface of leaves although they occur on both sides of many forms and on the upper surfaces of floating aquatic leaves. The openings are in the form of more or less narrow slits having the maximum measurements of .03 mm. in diameter, and an area of .0046 sq. mm., and a single leaf may be furnished with many millions of these organs.

271. Structure and Action of Stomata. Cut sections of the leaf of *Iris*, or *Amaryllis*, *Avena*, and *Caltha palustris* and note the structure of the stomata as seen in cross section and surface view.

[1] Darwin, F. Observations on stomata. Phil. Trans. Roy. Soc., **190** : 531. 1898.

Examine also the stomata of *Marchantia* or *Conacephalus* which are permanently open. Place leaves and thalli of the above species in the sun to wilt, and cut a thin surface section of the stomatal surface and examine dry. Compare the appearance of the stomata, with that seen in similar sections of fresh leaves. Place both kinds of sections in water and note results. If a number of species are examined it may be found that immersion in water will cause some stomata to close and others to open, owing to the different behavior of the epidermal cells. Take strips of epidermis from the lower surfaces of *Tradescantia discolor* standing
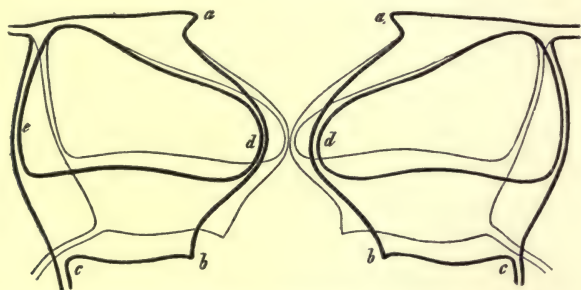
FIG. 99. Diagram of cross section of stoma showing the action of the guard cells. The heavy walls indicate the outlines of the guard cells when turgid and with the stoma open ; the thin walls indicate the contour of the guard cells when relaxed with the stoma closed. After Schwendener.

in sunlight and examine in water. Run a five per-cent. solution of cane sugar under the cover-glass and note result.

Any study of stomata in which their action is observed by means of a microscope will be vitiated with many errors, because in taking the epidermis from a leaf and mounting it for examination, stimuli are set up, which may cause the stoma to open or close before its original condition can be observed.

Practically all of the water given off by a leaf in transpiration passes through the stomata in the form of vapor, and the best method of ascertaining whether the stomata are opened or closed, is to use some means of detection of watery vapor. This may be done in two ways, *viz.*, by the cobalt method, in which paper

saturated with cobalt nitrate placed on the leaf changes from a bluish to a reddish color in the presence of watery vapor ; the second method consists in the use of a hygrometer. Several types of these instruments are in use in physiological laboratories. In one the variations in length of a strand of human hair with the changing humidity moves a lever carrying a pen which gives a constant record of the proportion of watery vapor in the air. This form has not been made suitable for testing the action of leaves. Another hygrometer consists essentially of an awn of some grass, like *Stipa*, which twists or untwists with the varia-tions in humidity of the atmosphere. This type has been found very useful in some forms of investigation. A third form con-tains a thin strip of some material which curves and straightens with the varying humidity, and the best example of this type is the horn hygrometer of F. Darwin, in which the sensitive mate-rial is made of a thin strip of pressed horn. The simpler forms of hygrometer sold in the market for general use have a sensi-tive strip composed of two layers of material of different hygro-scopicity, and the one described below is based upon this principle (Fig. 100).

272. Cobalt Test for Transpiration. Cut a few squares of mica 2 x 2 cm., such as may be in use for slips or covers in mounting algae. Saturate a few small pieces of good filter paper in a five per-cent. solution of cobalt nitrate, and dry thoroughly in sun-light, or in an oven. The transpiration of water from any given surface may be determined as follows : Cut a piece one cm. square from the prepared filter paper, dry for a moment over a gas flame, and lay on the surface of the leaf or other organ ; cover it with a piece of mica and seal the edges of the mica to the leaf by means of a wax composed of equal parts of beeswax, resin and vaseline. Open stomata and the excretion of watery vapor will be denoted by the change of the color of the filter paper from blue to reddish, which will take place in a few sec-onds. On the other hand, the color of the test paper will remain unchanged for days, perhaps, on surfaces free from stomata.

Preparations of this kind may be attached to a plant in several places, and it may be placed under different conditions of moisture, temperature, light, or the amount of moisture in the soil may be varied.

273. Use of a Differential Hygrometer. Construct a differential hygrometer as follows : Secure a piece of copper or iron wire 1–2 mm. in diameter and 25 cm. long. Thrust directly through the center of a cylindrical cork 1 cm. in diameter and 2 cm. long. Bend a section 4 cm. long at right angles and bring the cork near the bend on the long arm. Bend the wire again
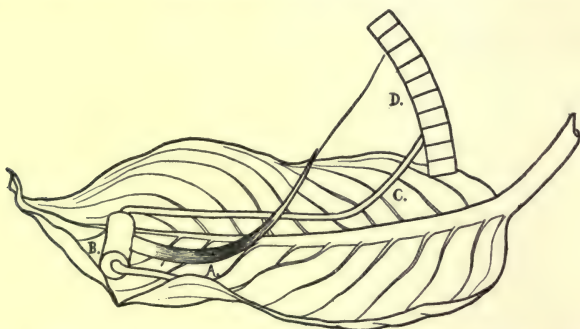
FIG. 100. Differential hygrometer. *A*, strip of film with layer of gelatine on upper side. *B*, cylindrical cork into which one end of the film is thrust. *D*, scale, over which the indicator has moved two divisions, showing open stomata, and transpiration, in the leaf on which the instrument rests.

at right angles beyond the cork and in the same plane as the first. Secure a film plate sold by dealers in photographic supplies, which consists of a thin sheet of celluloid coated with gelatine. Cut a strip 8 cm. long and 5 mm. wide, which will be curved owing to the contractility of the gelatine. Attach a stiff bristle or fiber to one end of the strip by means of glue, and thrust the other into the cork as in Fig. 100. Now bend the free end of the long arm of the wire so that it will support a curved scale made of paper. The gelatine of the strip is very delicately sensitive to all changes in atmospheric moisture.

Adjust the strip by turning the cork on its axis until the film

would lie within 2 or 3 mm. of the surface of a leaf on which it might be placed. Note the position of the pointer on the scale and set the instrument on a leaf laid flat on a table. If the lower surface of the leaf is uppermost, and the stomata are open, the film begins to straighten within ten seconds and the amplitude of the movement will correspond to the amount of watery vapor thrown off by the leaf. If the upper surface is tested no move-
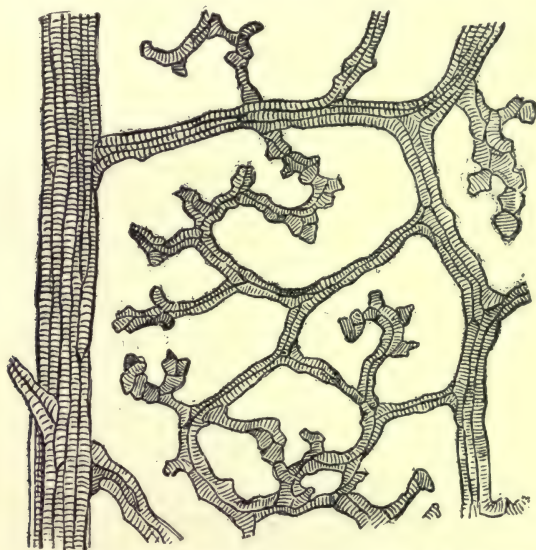
FIG. 101. Arrangement of elements conducting water in a leaf with netted veins. After Sachs.

ment will result unless open stomata are present. This test is a very delicate one, and vapor from the breath of the operator, or moisture from the hands may interfere with results unless care is exercised. Allow the index to return to zero after every test.[1]

274. Transpiration. The walls of all cells contain more or less water of imbibition, and when exposed to an atmosphere not saturated with watery vapor some of the liquid evaporates, a process which continues until checked by surface tension. Loss of water

[1] MacDougal. **A new hygrometer suitable for testing action of stomata.** Torreya, 1 : 16. 1901.

from the walls of cells in contact with the air is compensated by a current from the plasma, or from the walls of neighboring cells. By this last method of replenishing the loss, water, or solutions, may traverse the entire body of a plant without entering a protoplast or passing a single plasmatic membrane ; a small quantity only may be conducted in this way because of the great friction.

The outer membranes of the portions of plants exposed to the atmosphere are generally so cutinized, or impregnated with substances impervious to water, that they contain a small proportion of the fluid and hence evaporate but little into the air. Such loss of water through external membranes may be designated as cuticular transpiration. In leaves and other green parts of the plant the stomata connect directly with spaces among the cells containing air, and this air is in direct contact with thin-walled cells usually turgid, and with walls completely saturated with water. Evaporation goes on very rapidly and if the stomata are open the moisture-laden air in the intercellular spaces diffuses outward through the stomata and is replaced by air containing less moisture. The closure of the stomata allows the air in the intercellular spaces to become completely saturated, and hence transpiration ceases or diminishes, according to the completeness with which the pore is closed. This transmission of vapor through the stomata may be designated as diastomatic transpiration.

The loss of water from the cells exposed to the air either directly, or in the intercellular spaces, is replaced from the cells immediately below, or contiguous, in such manner that the loss is ultimately compensated by an equal amount taken in by the absorbing organs. This results in a more or less constant stream of water from the absorbing to the transpiring surfaces, which traverses the entire length of the body. The water absorbed contains substances from the substratum, generally mineral salts, necessary for the nutrition of the plant, which in this manner are carried through the body and brought within the osmotic influence of all of the living cells. A second important function of

transpiration consists in the facilitation of the gaseous exchange between the transpiring cells and the external air.

The leaves are the principal organs of transpiration, though this action is carried on by all tissues furnished with stomata, or lenticels, and to a slight degree by all surfaces of the plant as noted above.

Transpiration is affected by a number of external conditions, the most important of which are humidity of the air, temperature of the plant and the air, light, electric potential, air currents and
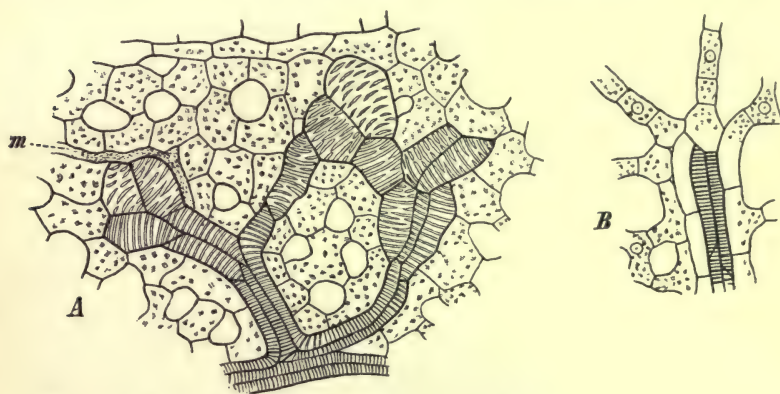
FIG. 102. Showing terminations of elements conducting water in leaves. *A*, in *Euphorbia splendens*, with a laticiferous tube at *m*. *B*, end of fibrovascular bundle and contiguous parenchyma cells in *Ficus elastica*. After Haberlandt.

mechanical vibration, amount of water in soil, and composition of the salts in the water absorbed.

On account of the above factors, the amount of water transpired by any given type of leaf, is much more in some localities than in others, which has resulted in the development of a large number of forms of transpiring organs with many adaptive structures. Many species are so plastic that the transpiratory conditions under which individuals are found are met by responses in the way of formation of leaves of a size and structure suitable to the environment.

Transpiration is also affected by a number of internal condi-

tions, among which the stage of development and differentiation of the tissues may be mentioned.[1]

275. Amount of Transpiration. Haberlandt found that a single individual of maize transpired 14 kg. of water during its development, which occupied 173 days, an individual of hemp 27 kg. in its development in 140 days, and a single plant of the sunflower the same amount during its development lasting the same length of time. Hales found that a sunflower having a total leaf surface of 9 sq. m. transpired .85 kg. in a single day. The amount of water transpired by a plant in any given period is generally about equal to the amount absorbed, provided the substratum furnishes a suitable supply of moisture. Two general methods may be used to estimate transpiration. By one method the plant is provided with a supply of water, and set on a balance so that the amount of water lost, can be detected by the loss in weight. The second method entails the measurement of the amount of water taken up under conditions, which must be uniform for some time before the beginning of the test. Still a third method of limited usefulness consists in enclosing the plant in a closed vessel containing a known weight of some substance that will absorb watery vapor from the air. The increase in the weight of the absorbent denotes the amount of transpiration.[2]

276. Determination of Transpiration by Weighing. Provide a plant with large leaf surface growing in a 3-inch pot and set it in a tin pail slightly larger and deeper, and containing a few cc. of water to keep the soil moist. Tie a piece of oiled cloth over the top of the pail and tightly around the stem to prevent the escape of watery vapor. Bring the edges of the cloth down under the pail and fasten. The whole preparation should not exceed 800 g. in weight. Weigh exactly on a balance of suffi-

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[1] For complete bibliography, see Burgerstein. Materialen zu einer Monographie d. Transpiration d. Pflanzen. I. 1887. II. 1889. III. 1900.

[2] Kohl. Transpiration. 1886.

Pfeffer, W. Physiology of Plants, 228. 1900.

Copeland, E. B. A new self-registering transpiration machine. Bot. Gazette. 26 : 243. 1898.

cient capacity, and which is accurate to .5 g.   Set in bright sun-
light near a window or in a glass experiment room, and place a
thermometer, or better a thermograph, near the preparation.
Four hours later again weigh the preparation and note the total
loss of weight.   This will represent the amount of water given off
by the stems and leaves during the period over which the experi-
ment extended.   Still more reliable results may be reached if
the test is continued during 8 or 10 hours.   Strip the leaves from
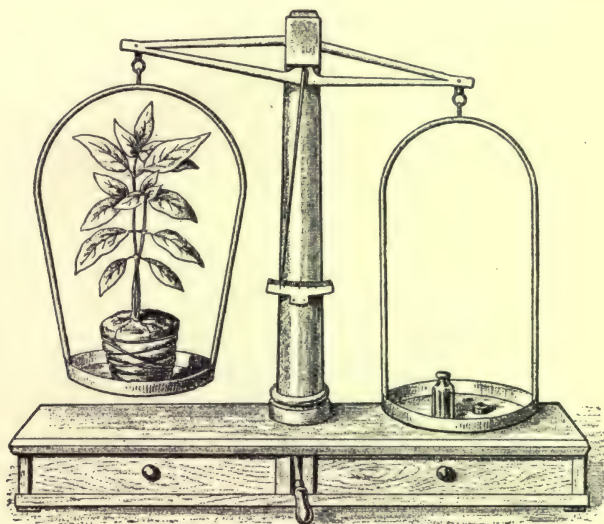
FIG. 103. Balance for determination of amount of transpiration.   After Giesenhagen.

the stem and place together on a table, matching the edges to
form a regular rectangular figure, the area of which may be easily
calculated.   A more accurate method consists in tracing the
outlines of the leaves on a sheet of paper of known area.   Weigh
the paper on a precision balance, then cut out the figures of the
leaves, weighing remainder.   Comparison of the data will give
total area of leaves, or find the area of the leaf-tracings by the use
of a planimeter.   Compute the area of transpiration per square
centimeter of leaf surface (Fig. 103).

277. Comparative Amount of Transpiration of Stems and Leaves.
The preparation used in the last experiment should be set aside, and
on the following day should be weighed at the same hour on which
the previous experiment was begun, and again 8 or 10 hours
later. Note the total loss of weight and compare with that of
previous test. Deduct from total of previous day and obtain cor-
rected rate. Compute rate per square centimeter of surface of
stem. A small plant of sunflower will be found suitable for this
experiment. When the leaves are taken off, it should be done
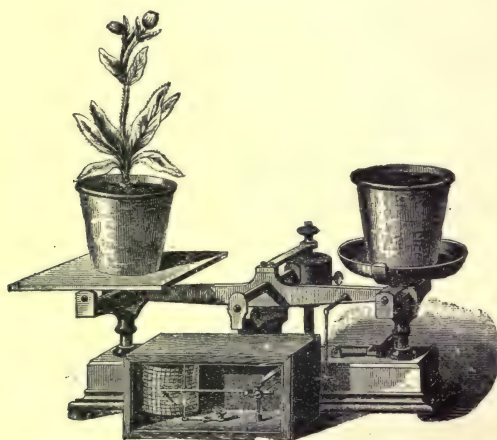
FIG. 104. Recording balance, with pot of earth on one pan and plant on the other.
The difference of loss of weight of the two is traced by a pen on the cylinder, moved
by clockwork beneath.

by cutting the petioles neatly near their bases, and applying gela-
tine to the excised surfaces. Guttation if present will invalidate
the results.

278. Influence of Light on Transpiration. Make a preparation
as in 276 and obtain the rate of transpiration during a period
of four hours in bright sunlight, using a thermograph and hygro-
graph, to make a continuous temperature and humidity record.
Now set the plant in a dark room and keep similar records dur-
ing an equal period. If the temperature and moisture are equal,

a direct comparison of the influence of light and darkness may be made.

279. Determination of Amount of Transpiration by a Potometer. If the negative pressure in a plant is equalized the amount of
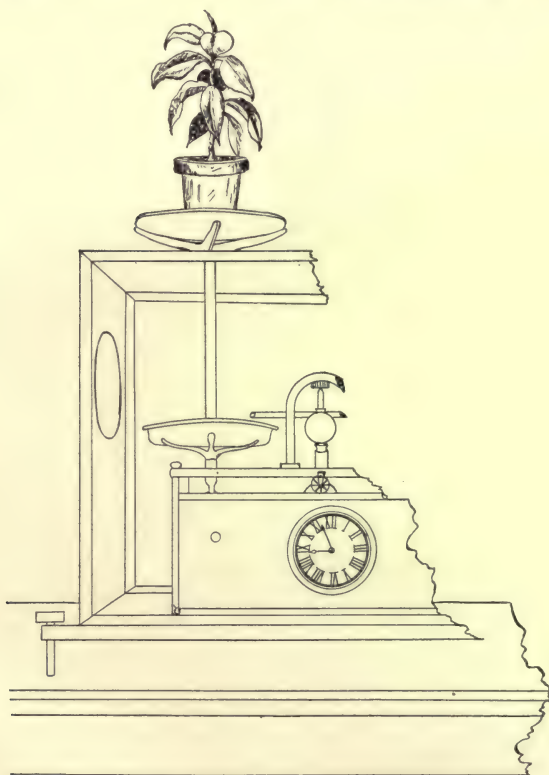
FIG. 105. Determination of amount of transpiration by Anderson automatic balance. The loss of weight of the plant is equalized by weights, which are added and recorded automatically.

water taken up in any given time, is a fair equivalent of the amount of transpiration during the same period. It is possible to make much more accurate measurements of the water taken in than that given off, by ordinary methods, and the potometer is perhaps the most useful instrument in studying transpiration. The fol-

lowing apparatus has been designed by the author and tested by several years' use.   A capillary tube is graduated into several portions containing 100 mg. of water, and a length of 1 cm. at one end is bent downward at right angles, while a double length is bent into U form at the opposite end.   To this U is attached a 3-way tube by means of rubber tubing wired.   The upper end of the 3-way tube is attached to a separatory funnel by means of a rubber tube, or it may be fused on permanently.   The long calibrated tube is supported on a wooden base and the 3-way tube by means of an iron post driven in the base.

To determine the amount of water used by a shoot, fill the separatory funnel and all of the tubes with water.   Select a fuchsia, geranium or any woody-stemmed plant and lay it in an aquarium, or tank of water, and cut off the stem in such manner that the negative pressure will carry water up into the vessels after the excision is made (Fig. 106).   It may be necessary to allow the shoot to stand in the water for a day before being fitted to the apparatus.   Fasten a section of rubber tubing to the free end of the 3-way tube and wire it.   Trim the base of the stem obliquely. Now open the stopcock of the funnel and allow water to run slowly out of the tube to which the branch is to be fitted.   Insert the base of the stem in the section of rubber tubing, taking care that no air bubbles are included, and wire tightly.   Clear the tubes of all air bubbles, and set a small bottle under the end of the capillary tube at B.   Place a thermometer near the plant, and have a watch convenient.   Allow a small bubble of air to enter the tube, and as the transpiring plant withdraws water from the system of tubes, the bubble will traverse the capillary tube.   Mark the number of minutes, or seconds, necessary for the bubble to travel through each section containing 100 mg. and when it has passed the last calibration turn the stopcock, and force it back beyond the first mark and repeat as often as desirable.[1]

The influence of negative pressure on such tests is illustrated by the following experience.   A leafy shoot of fuschia in which

[1] MacDougal.   A convenient potometer.   Bot. Gazette. **24** : 110.   1897.

a negative pressure existed was cut off under water at 12:45 P. M. and fastened to the apparatus at 1 P. M. After a few minutes the following observations were made :

1:27	P. M.	Indicator bubble at			0 mg.	Temp.	21.0° C.
1:50	"	"	"		300	"	21.0
2:05	"	"	"		500	"	21.0
2:14	"	"	"		600	"	21.0
Readjusted.							
2:17	"	"	"		0	"	20.8
2:28	"	"	"		100	"	20.7
2:40	"	"	"		200	"	20.5
2:57	"	"	"		300	"	20.0

This shoot was taken from the apparatus and placed in water. The following day a small portion was cut from the excised end, and it was refitted to the apparatus with the following results, which show that the negative pressure had been equalized during the first day :

11:13:55	A. M.	Indicator bubble at			0 mg.	Temp.	20 0° C.
11:26	"	"	"		100	"	20.0
11:39	"	"	"		200	"	20.1
11:53	"	"	"		300	"	20.4
12:05:5	"	"	"		400	"	20.7
12:20	"	"	"		500	"	21.0
12:33	"	"	"		600	"	21.0

The leaves showed a superficial extension of 300 sq. cm., including the petioles ; area of stem surfaces, 40 sq. cm. This apparatus is also convenient for obtaining the comparative transpiration of the stems and leaves. The leaves were stripped from the shoot of the above tested plant, the base of which was trimmed and refitted to the apparatus, and the following observations were made :

10:31	A. M.	Indicator bubble at			0 mg.	Temp.	17.5° C.
11:12	"	"	"		100	"	18.0
11:43	"	"	"		200	"	18.8
12:13	P. M.	"	"		300	"	17.5
1:23	"	"	"		500	"	16.0
2:00	"	"	"		600	"	15.1

280. Force Exerted by Transpiring Shoots. Cut off a branch or stem of any woody plant and fit to the end of a glass tube of

15

the same external diameter by means of a short section of rubber tubing secured by wound and twisted wire.   Invert the tube and fill the tube with water which has been boiled, and use other precautions to exclude air.   Place the finger over the end of the tube and set upright in a small dish of mercury.   As the shoot uses water it will withdraw it from the tube and raise a column of mercury.   Note the height of the column at intervals of 12 hours for a day or two.   The lifting of the mercury will continue until
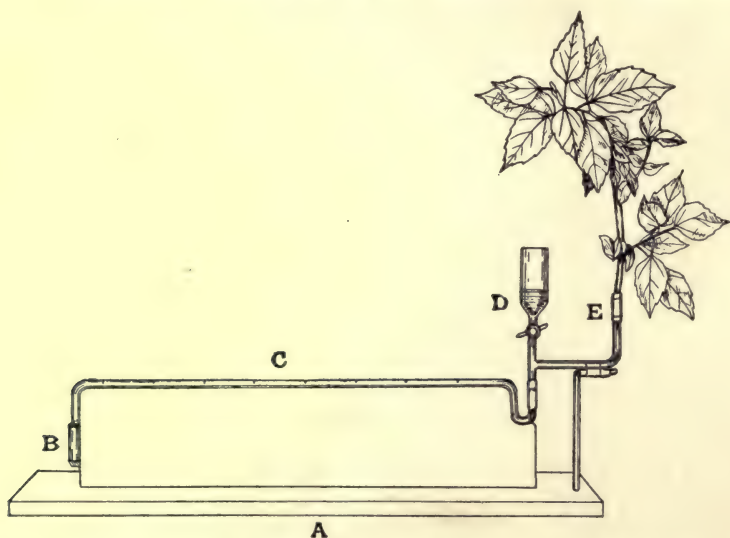
FIG. 106.   Potometer.   *A*, base.   *B*, reservoir for water.   *C*, calibrated tube.   *D*, separatory funnel for water supply.   *E*, fitting of plant and tube.

its weight is sufficient to pull air down through the stomata and intercellular spaces of the cortex into the tube, where it will gather at the lower end of the branch and prevent absorption.   It is thus to be seen that the experiment does not measure the full lifting power of transpiration, which often exceeds 6 to 8 atmospheres.   The greatest lifting power may be demonstrated in species furnished most sparingly with intercellular air spaces.   The action will be found most rapid in *Eucalyptus* and similar forms, and most enduring in stems furnished with cladodes, and phyllodes instead of

typical leaves.   Active coniferous shoots have been found to exert
a force sufficient to raise a column of mercury 76 cm. (Fig. 107).

**281. The Recovery of Wilted Leaves by Aid of Increased Pressure
in Stems.**   Cut a young shoot of *Helianthus* and allow it to be
supported in the air until the leaves have
wilted noticeably.   Now fasten it to one
arm of a U-tube and fill the tube with
water.   If recovery does not take place
pour several cc. of mercury in the free end
of the tube.   This will drive the water up
into the stem with some force and will re-
sult in the recovery of the drooping leaves
(Fig. 108).
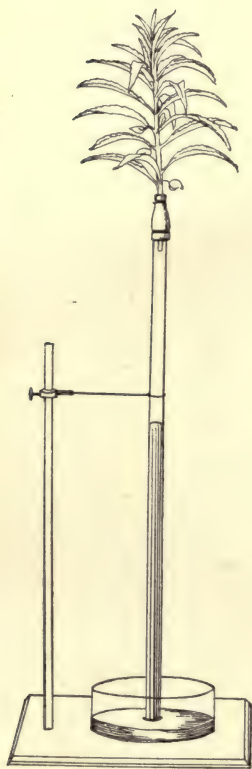
FIG. 107.   Demonstration of lifting power of transpiration by a shoot attached to a
tube filled with water and standing in a dish of mercury.

FIG. 108.   Shoot attached to short arm of U-tube, into which water and mercury
are poured to illustrate influence of pressure upon absorption and consequent recovery
from wilting.   After Sachs.

282. Path of Sap Through Stems.   The function of conduction
of solutions, absorbed by the roots, in small annual plants, is car-

ried out with no great physical difficulty, and no special differen-
tiation of tissue for this purpose is necessary.   When the leafy
crown to which the current of water must be led is separated
from the roots by a distance of many meters, on a huge trunk,
mostly composed of dead cells, special provisions may be looked
for to ensure the proper supply of water to the transpiring sur-
faces.   The upward movement of sap takes place through the
xylem, and for the most part through the vessels and tracheids.
In woody stems of perennial plants, such as trunks of trees, the
movement is largely through the most recently formed layers,
and is most rapid in the elements formed in the spring or begin-
ning of the active season of growth, the mature wood partly losing
its capacity to transport water with age.   Trees with a large
amount of sap wood, or alburnum, carry the water supply up
through a comparatively thick cylinder of wood, and hence must
be girdled deeply to be killed.   The beeches and birches are
examples of this kind.   On the other hand the wood of the oaks,
pines and cherries ages rapidly with respect to this capacity, and
a shallow girdling of such trees will cause death because of the
incapacity of the older wood to carry an adequate supply of water
to the crown.   The path of sap through stems may be demon-
strated by allowing the plant to absorb and carry up reagents
which will stain the elements through which they pass or be
easily detected by chemical reactions.

An earlier theory as to the method of the ascent of sap sup-
posed that fluids were conducted from the roots to the shoots
through the walls of the elements concerned only, and that the
lumina of the cells were empty or served as reservoirs for sur-
plus supply of the liquid.   A certain amount of water undoubt-
edly does traverse the entire body of the plant along the walls,
but the quantity which might be conveyed from the roots to
the shoot by this path would be wholly inadequate to meet the
needs of actively transpiring leaves.   That the current of water
from the base to the crown traverses the cavities of the vessels
and tracheids seems to be conclusively demonstrated by the fact

that mechanical compression of the stem, which would partially close the cavities, lessens the transpiration stream.

283. Demonstration of Path of Sap.   Cut off a shoot of *Impatiens* and place in water for an hour to equalize the negative pressure, if it should exist.   Now place the lower cut end of the shoot in a beaker containing a saturated solution of eosin in water, and stand in diffuse light.   Note the appearance of the dye in the stem a few hours later.   Cut sections of the stems and determine elements affected by the stain.   It is to be kept in mind that the dye is carried upward in certain elements, but that it also slowly diffuses laterally and may be found after some time in cells not concerned in the transportation of water upward.   This test may also be made with *Zea*, *Helianthus*, or any species of the mint family.

284. Comparison of the Capacity of Old and New Wood for Conducting Water.   Place a branch, which has been cut from an oak or cherry tree, in water for a time to equalize the negative pressure.   Now immerse the base of the shoot in an eosin solution and note the region through which the fluid is carried most readily.   Care must be taken not to injure the bark on the base of the branch, so that it may absorb only by the cut surfaces.

Select another branch and make a microscopical examination of the stem with the view of finding differences between the wood which conducts water readily and that which does not.   Make longitudinal sections and note thickness of wall, character of pits, and chemical properties of wall with especial respect to lignin.[1]

285. Rate of Ascent of Current of Water through Stems.   The dye used in the above experiments does not readily pass through plasmatic membranes, hence the roots of the plants on which tests were made were cut away so that the solution might be taken up directly by the dead elements.   If salts are used which may be taken up by the root hairs the entire plant may be used in the experiment.   Advantage may be taken of this fact in the demonstration of the rate at which solutions travel upward through stems.

[1] For lignin test see Zimmermann's Botanical Microtechnique.

Place a few large actively growing specimens of *Helianthus* in sunlight at a suitable temperature, and clear away the top layers of soil so that solutions poured in the pots will quickly reach the roots.   Instead of the usual morning supply of water given to the plants pour in .3 liter of 2 per-cent. solution of lithium nitrate. An hour later cut off the stem and divide the leaves and shoot into a number of sections, preserving their relative positions.   Now beginning with the topmost section, burn it in a flame before a suitable spectroscope and look for the characteristic spectrum of lithium, which is characterized by a brilliant carmine red line between *B* and *C* at 32.   Larger quantities of the metal in the tissue will give a carmine red tint to a colorless bunsen, or blowpipe

FIG. 109. Showing path of water from vessels to cells of leaf of *Impatiens parviflora*.   After Strasburger.

flame.   This may be seen best through a sheet of blue glass. Continue this until the presence of the metal is detected.   Measure the distance from the section in which it was found to the roots in which it might have been absorbed, and calculate the rate per hour.   The two sources of error in this test consist in the time necessary for the solution to be taken up by the root hairs, and also in the fact that the salts of lithium and other substances as well are not carried upward through the stem as rapidly as weaker solutions or water would be.

Cut off stems of the same species, and place the lower ends in solutions of eosin and compare the rate of conduction indicated, with that determined by the lithium reaction.   Negative pressure should be equalized before the cut stems are placed in the colored solutions.   Extremely rapid conduction will be found in the

Cucurbitaceae. *Bryonia* and *Cucurbita* are found to show a rate of 6 meters per hour. The rate is probably between one and two meters per hour in ordinary broad-leaved trees. In other species the rate may be as low as .18 meter per hour.

286. Mechanism by which Water is Conducted Upward through Stems. Information concerning the factors operative in the conduction of sap from the roots to the crowns of tall plants is most incomplete and unsatisfactory, and no adequate explanation may be given of the phenomena involved.

Only two sources of energy are known by which water may be lifted from the soil to the crown of a tree : the force set free in evaporation, and the osmotic action of the cells, from which the transpiration has withdrawn water. The evaporation of water from the walls of cells in leaves might be replaced by water of imbibition drawn up the entire length of the plant in the wall ; it would be utterly impossible to carry upward the enormous amount of water actually thrown off by the plant in this manner, however. Evaporation from the walls of a turgid cell in a leaf would be replaced by liquid withdrawn from the cell sap. The cell sap would thus be rendered more concentrated and would set up a chain of osmotic attractions reaching to the tracheids. The osmotic attraction of the sap of transpiring cells is capable of exerting a force of 6 to 8 atmospheres, which would be sufficient to lift water to the tops of tall trees if the liquid were in the form of a solid vertical column. The tensile strength of the solid column would prevent it from breaking if it were continuous, but the column passes through the cavities of the box-shaped tracheids, and exists in the cross walls as water of imbibition. Furthermore the cavities of the tracheids are partly filled with air bubbles of varying size, which would tend to weaken the cohesive power of the column. The physical properties of the column under such conditions are not easily predicated. The theory of Westermaier that water is forced upward from one level to another by the action of the living cells of the medullary rays is found to be entirely unsupported, but interference with the as-

cending current undoubtedly takes place so that it may not be considered as a simple, upwardly moving stream of water.[1]   In a series of experiments by Dr. C. C. Curtis in the laboratories of the New York Botanical Garden, a number of manometers were attached to the lateral branches of small trees of *Populus Simoni* at distances of 20 cm. to 5 m. from the roots.   Regions of positive and negative pressure were found variously distributed in the stems, and in some instances the only positive pressures found were at the extreme tip of the shoot.   It is evident, therefore, that the conduction of water from the roots to the leaves may be most seriously influenced by the osmotic absorption, and exudation pressure, of the layers of living cells lying along the pathway of the ascending current, and the probability is by no means excluded that these may be principal factors in the conduction of water from the roots to the crown : a probability by no means lessened by the fact that the upward current may traverse long portions of the stem, in which all of the living cells are dead.[2]

[1] Dixon and Joly.   Phil. Trans. Roy. Soc. **186** : 1895.
Dixon, H. R.   Proc. Roy. Soc. **4** : 1898.
[2] See, Pfeffer, W.   Physiology of Plants. **1** : 220–227.   1900.   And Ward, H. M. Timber and some of its diseases. **59–141**.   1897.

XI. NUTRITIVE METABOLISM

287. Essential Constituents of the Food of Plants. A careful chemical control of the medium in which a plant lives and the substratum to which it is attached, demonstrates that the elements necessary for growth and existence, comprise carbon, oxygen, nitrogen, hydrogen, sulphur, phosphorus, calcium, potassium, iron, and magnesium. Calcium, however, is not necessary for the fungi. The supply of some of these elements may be partially replaced by others which may be themselves essential or non-essential. Thus, for instance, it is found that sodium and calcium may partly fill the place of potassium and magnesium under some conditions of growth. The analysis of the bodies of plants reveals the fact that many other substances are often present, and it is to be said that almost any element in the soil may be taken up in such quantity as to form a noticeable proportion of the ash.

The presence of sodium and chlorine in the substratum is often an important condition : although these substances may not be actually used in the plant, yet they exert a tonic influence upon it by stimulating the absorbing organs, and play an important part by their chemical action on the other constituents of the substratum (97).

Carbon is obtained from the carbon dioxide of the air by green plants and from organic compounds by chlorophylless forms. This element is perhaps the most important as it enters largely into all compounds from which the organism is constructed.

Oxygen is a constituent of the combustible substances of the plant, and is obtained from the air, water, salts and oxides taken in from the soil. Hydrogen is absorbed in the form of water by green plants, in the form of ammonia and its compounds, and sparingly in the form of complex compounds by the higher plants, which form a large proportion of the food of the bacteria and

fungi.   It accompanies carbon in the construction of the more
important compounds of protoplasm.

Nitrogen is generally absorbed from the soil in the form of
nitrates and ammonium salts by green plants, although phanero-
gams and algae may absorb minute proportions of such substances
as urea, glycocoll, asparagin, leucin, tyrosin, guanin, kreatin, hip-
puric acid, uric acid, acetamide, and propylamine, and obtain some
nitrogen in this manner.   Some independent organisms inclusive
of *Clostridium* and bacteria of leguminous tubercles have the
power of fixing the free nitrogen of the air.   Nitrogen enters into
the composition of the proteids, and is equally important with
carbon in the construction of living matter.

Sulphur is absorbed in the form of sulphates and enters into
the composition of many proteids, and some volatile oils.

Phosphorus is absorbed in the form of the phosphates, and it
may also be taken up in some of its organic compounds such as
lecithin.   It enters into the composition of nuclein, and plastin
which have an important place in the organized structure of the
cell and also enters into lecithin, the function of which is in some
doubt.

Calcium is absorbed in the form of the phosphate, nitrate, sul-
phate and carbonate, undergoing decomposition in the last named
compound during the process.   Calcium is not found in embry-
onic tissues, but is abundant in adult cells in which, especially, it
is infiltrated in the wall.   It is difficult to attribute any direct
function to this element ; although its absence occasions serious
disturbances in the higher plants, yet fungi may carry on normal
development without it.   The presence of calcium salts in a cell
results in the formation of insoluble precipitates when oxalic acid
is formed, which is probably one of the important uses of this
element.   Its connection with pectic acid in forming cell-mem-
branes may prove to be its most important purpose.

Potassium is absorbed as sulphates, phosphates, silicates and
chlorides.   It is abundant in embryonic tissues, is found associ-
ated with reserve food and material in transit.   It is also possible

that it takes a part in the processes of formation of proteids, carbohydrates and fats. It sustains an important physical function in the maintenance of turgidity.[1]

Magnesium, is taken up in all of its salts except the chloride and is found in globoids, occurring in the greatest abundance in seeds, also in embryonic tissues. It is concerned in the synthetic processes although its exact office may not be delimited.

Iron may be absorbed in almost any of its salts, but is used only in minute quantities. It is present both in the plasma and wall and may enter into some organic unions. In this form it may aid in the construction of chloroplasts. Its presence is necessary for the formation of chlorophyl although it does not enter into chemical union with this compound. It is equally indispensable for species which do not construct chlorophyl.[2]

288. Structure and Arrangement of Living Matter. A cell or protoplast is a minute mass of protoplasm which tends to assume a globular form, but which undergoes such modifications by growth, differentiation, internal movement and mechanical pressure that it may assume almost any form from spindle-shaped, tabloid, etc., to globose. Protoplasm is a viscid translucent substance, which in some instances appears to consist of a meshwork or reticulum enclosing a ground substance (hyaloplasma). The reticulum shows more or less abundant rounded bodies on its branches (microsomes), granules, and imbedded in the mass are numbers of inert substances such as crystalloids, giving rise to the "fibrillar theory" of the structure of living matter. To other observers protoplasm has appeared to show a foam structure consisting of minute closely crowded drops of "alveolar substance" imbedded in another liquid constituting an emulsion (alveolar theory of Butschli). Protoplasm is supposed by some investigators to consist of innumerable minute granules which form its essential basis,

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[1] Copeland, E. B. The relation of nutrient salts to turgor. Bot. Gazette **24**: 399. 1897.

[2] Pfeffer. Plant Physiology. **1** : 410. 1900.

Loew, O. Physiological role of mineral nutrients. Bull. No. **18**, U. S. Dept. of Agric., Division of Veg. Path. and Physiol. 1899.

and other arrangements with these are but of secondary importance (granular theory of Altmann).[1]

A denser globose body, generally with a definite limiting membrane, lies imbedded in the cytoplasm constituting the nucleus. The essential part of this organ appears to consist of an irregular branching network composed of *linin*, a granular substance resembling extra-nuclear protoplasm in its chemical composition and granular appearance, and *chromatin* a deeply staining substance, which often appears as masses or granules imbedded in the linin or may be separated in a single mass constituting the nucleolus. In addition a clear substance occupies the interspaces of the network. Numbers of granular bodies of definite shape termed plastids are also to be found at various points in the cell. The protoplasm immediately surrounding the nucleus appears to be denser, and less differentiated morphologically, than the outer portions of the mass, and these two regions may be denoted as the endoplasm and ectoplasm respectively. Large spaces, containing clear solutions or various substances, appear in the plasma and are termed vacuoles. The ectoplasm is limited by a distinct membrane, and lies against the outer wall which makes up such a large share of the visible portions of plants. The wall may show the most diverse forms and thicknesses and is generally regarded as a secretion product of the living substance, and many changes may be induced in it at all stages of its existence both as to structure and composition.

289. Chemical Properties of the Cell. The nucleus and cytoplasm have some constituents in common, but the former is characterized by the fact that it is chiefly composed of nucleins and nucleo-proteids in addition to the nucleo-albumins, globulins, albumins and peptones of which cytoplasm is largely made up. Chromatin is composed almost wholly of a compound of nucleinic acid ($C_{40}H_{54}N_{14}P_4O_{27}$) and certain proteids, while the linin is composed of proteids readily soluble in acid pepsin. The proteids of the cytoplasm show the more diverse characteristics and the

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[1] Wilson, E. B. The cell in development and inheritance, p. 17. 1900.

groupings of the components of these substances vary sufficiently to give the widest chemical, physical and physiological properties. No definite information is at hand concerning the relative composition of the cytoplasm and the plastids lying in it. The latter are probably of a denser consistency. Neither is the character of the plasmatic membrane known. The cell wall is a secretion product of the protoplast, and is not to be regarded as living substance even in its earlier stages of formation, although it is at all times during the life of the cell under the control of the living matter, so far as its form and structure are concerned. The wall may differ widely in structure and chemical composition. It is composed of a group of carbohydrates which for convenience may be grouped under the term cellulose. The presence or proportion of any of the constituents is a matter of the greatest variation. The wall is in a state of constant change during the life of the protoplast which it encloses. Not only does the living matter induce changes in it but the infiltration of the material drawn into the cell, and the deposition of other secretions, add to the complexity of the wall which is thus seen to be a morphological affair, rather than an organ of the cell. Lignification, suberization, cutinization and the formation of pectates as a result of the action of certain enzymes (see pectase) are the more important of such changes.

290. Functional Relations of the Cell Components. A protoplast may be regarded as a physiological, or functional unit, and none of its organic components are capable of anything but limited existence, and restricted action, when separated from the remainder of the cell. The nucleus or fragments of cytoplasm, may live for many days or even weeks, when separated from each other, and many forms of metabolism may be carried on in them, but no actual independence or complete action is established. Some writers maintain that separated fragments of the protoplast can carry on only destructively metabolic processes and the functions connected with them, but this is not confirmed by all of the facts. Thus the synthesis of carbohydrates is accomplished by separated

chloroplasts, and a careful examination would doubtless bring many other instances of the same kind to light.

Furthermore it is not to be taken for granted that any function of the cell is the specific action of the organ in which the results of the process become manifest. Thus the wall apparently secreted by the cytoplasm, is not formed in the absence of the nucleus, which may not lay down this membrane by its own separate action. Again the assumption that the nucleus is the seat or especial organ of the synthetic processes, and the cytoplasm the arena for the liberation of energy may not be maintained in view of the facts just related. Nor can the function of nutrition, or even absorption be ascribed to any region of the cell. While the absorption of material must take place through the outer plasmatic membrane yet the energy for the attractive process is furnished at some distance from it, and in fact the whole cytoplasmic mass may be regarded as an osmotic membrane.[1]

The components of the cell are therefore to be regarded as mutually interdependent physiologically, although it is to be conceded that the nucleus occupies a directive position in all morphological constructive operations of the protoplast, a familiar instance of which is to be seen in the unequal thickening of cell walls according the proximity of the nucleus. Furthermore it is supposed that the nucleus is a primary factor in transmitting the qualities of the species from one individual to another in lineal succession, yet the action of the cytoplasm has not been wholly excluded in any experimental evidence hitherto offered.

291. Nutritive Elements Obtained from the Soil by Green Plants. A determination of the elements taken from the soil by green plants may be made by cultures in which plants are grown in some neutral substratum like quartz sand, or distilled water, to which is added solutions of the various substances to be tested. The results of such investigations are often obscure, owing to

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[1] See conflicting views, Wilson, E. B. The cell in development and inheritance, p. 341. 1900.
Pfeffer, W. Physiology of Plants. 1 : 50. 1900.

the great number of possible vitiating circumstances.   The me-
chanical qualities offered the plant are generally quite different in
such tests from the substratum afforded by natural soil, and it is
difficult to imitate the composition of
the soil solutions.   The seasonal con-
ditions are also generally reversed during
the periods in which such work is at-
tempted in laboratories, and the amount
of substances already present in a cutting,
or seed, tends to lessen the definiteness
of the results in the exclusion of any
element from the culture solutions.
Lastly it is to be said that only a
majority of the experiments will succeed
even under the best care and most

FIG. 100. Top suitable for
the support of plants in water
cultures.   After Detmer.

favorable conditions, so that all of the tests described below
should be performed with many separate individuals.

200. Water Cultures.   Secure a number of glass jars of a ca-
pacity of about 2 liters, and provide for each, tops of wood or
earthenware which are made with a slot extending from one side
to the center, joining a hole made to receive the plant (Fig. 100).
Another hole may be made which will hold a stick or rod for the
support of the plants.   Germinate a number of seeds of any com-
mon plant such as *Phaseolus, Triticum, Avena, Zea,* or *Convolvulus,*
by placing them in a suitable germinator, or between folds of damp
cloth.   When the roots have attained a length of a few centimeters
remove and clean carefully.   To place the plant in proper position
for the culture test, it should be set upright in the central open-
ing of the top, and held in place at first with asbestos fiber, or cot-
ton wool, wedged loosely around it, taking great care that the
young stem is not bruised in the process.   As it grows it may be
held to the wooden support by means of cords.   The plant should
be placed so that the roots only will depend on the fluid in the
jar, and care should be taken that the packing around the stem
is kept dry.

Before placing in position the jar should be cleaned with nitric acid, and washed out with distilled water, then rinsed with corrosive sublimate, which should also be thoroughly washed out with distilled water. The culture solution should be poured in until it reaches to within 2 cm. of the top. The conditions for light and temperature may be made fairly normal if the jars are set deeply in a large box containing soil. The plants should be removed from the jars every ten days and the latter should be rinsed out with distilled water and refilled with fresh solution. During this process the plant, which is attached to the top, should be set over a similar jar containing distilled water. The temperature of the air should be made suitable for the species tested.

The plants should be grown in a solution containing all of the indispensable elements, and in others from which certain ones are lacking. The different solutions may be made up as below, and kept in tightly stoppered bottles, in darkness, and then diluted with distilled water in the proportion of 10 parts of the solution to 48 of water. The following solutions are those used by Schimper[1] and will suffice to carry a single series of simple tests. It will be found possible to carry the plant to maturity in the normal solution and this should be done if convenient, for the purposes of comparison with those grown in incomplete solutions.

Normal Solution.

6 g. calcium nitrate.
1.5 g. potassium nitrate.
1.5 g. magnesium sulphate.
1.5 g. neutral potassium phosphate.
1.5 g. sodium chloride.
600 cc. distilled water.

Solution Lacking Calcium.

7 g. potassium nitrate.
1.5 g. magnesium sulphate.

[1] Schimper, A. F. W. Zur Frage der Assimilation durch die grüne Pflanze. Flora. **73**: 207. 1890.

1.5 g. sodium chloride.

1.5 g. neutral potassium phosphate.

600 cc. distilled water.

Solution Lacking Potassium.

7 g. calcium nitrate.

1.5 g. magnesium sulphate.

1.5 g. sodium chloride.

1.5 g. neutral sodium phosphate, or calcium phosphate in excess.

600 cc. distilled water.

Solution Lacking Magnesium.

6 g. calcium nitrate.

1.5 g. potassium nitrate.

1.5 g. neutral potassium phosphate.

1.2 g. potassium sulphate or an excess of calcium phosphate.

600 cc. distilled water.

Solution Lacking Nitrates.

1.5 g. neutral potassium phosphate.

1.5 g. magnesium phosphate.

1.5 g. potassium chloride.

600 cc. distilled water.

Solution Lacking Phosphates.

0.5 g. potassium nitrate.

1 g. calcium nitrate.

0.5 g. magnesium nitrate.

0.5 g. neutral potassium sulphate.

1,000 cc. distilled water.

This solution is to be used without the addition of more water.

A drop of solution of iron chloride should be added to the culture jar every time it is filled or refilled. The chemicals used should be absolutely pure. A daily aeration of the solutions in the jars by means of a blower attached to a water tap, or an aspirator will be an advantage (Figs. 92, and 111). The solutions in the stock bottles should be well shaken up before the necessary

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amount is measured out for refilling the jars, since some of the calcium salts used are only sparingly soluble, and will collect at the bottom of the vessel.

Cuttings of *Salix, Begonia*, or any convenient plant may be used instead of seedlings. Half of the length of the cutting should be immersed in the solution, and it should be cared for otherwise, as a seedling.

Successful water cultures of aquatic plants have been carried out with *Lemna* and *Philotria*, but solutions of less concentration should be used. A definite number of fronds of *Lemna* should be placed in an open vessel or small glass aquarium under proper conditions for at least six weeks and then the multiplication of the individuals noted. Shoots of *Philotria* should be measured and roughly sketched, then cultivated for a similar length of time in an open vessel containing the solutions. A second measurement and sketch will afford a comparison which will determine the effect of the substances tested.
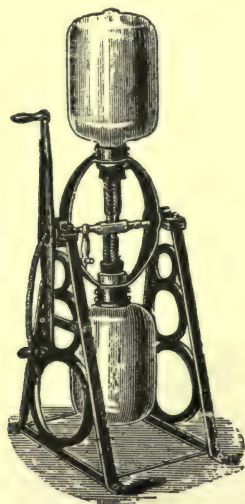
FIG. 111. Aspirator.

293. Absorption and Use of Carbon. The air contains about 28 parts carbon dioxide in 100,000, and all natural waters and soils are more or less saturated with it. As a consequence of this universal distribution, and of the fact that it is constantly liberated in the tissues as the result of respiration, the sap of the plant always contains some of this substance which is slowly diffused outwardly. In cells containing chlorophyl, and perhaps etiolin, however the carbon dioxide is subjected to forces which disintegrate it and set free a portion of the oxygen which it contains. This excretion of oxygen and decomposition of carbon dioxide takes place only when the plasma containing the chlorophyl is exposed to light. The energy necessary to accom-

plish the breaking up of the carbon dioxide molecule is presumably derived from the radiations absorbed. The remainder of the molecule of carbon dioxide, with its unsatisfied chemical affinities, is further acted upon by protoplasm of the chloroplast, and as the result of the synthetic processes a carbohydrate, generally cane sugar, is formed.

The successive steps in the construction of this complex compound, and the part played by the plasma are wholly unknown, and even the outline given is based in great part upon theoretical considerations.[1] The entire process is known as photosynthesis. The products may be diffused a rapidly as formed, and undergo further combination to form nitrogenous bodies, or they may accumulate because of the great activity of the chloroplasts. The accumulation may appear as glucose in cells of some plants, although in the greater number of species the saturation

FIG. 112. Cultures of hemp in neutral solid substratum. A complete nutrient solution has been added to I, and the plants have attained a height of 1.5 meters: a solution lacking potassium nitrate has been added to the substratum in II, and only the sterile substratum placed in the pot in III. After Ville.

of the cell with the photosynthetic products is followed by their condensation into some insoluble form as starch. During the day the action of light causes the accumulation of the surplus products, which are more or less completely translocated from the green cells during the succeeding period of darkness.

[1] Went, A. F. C. Chemisch-physiologische Untersuchungen ueber das Zuckerrohr. Jahrb. Wiss. Bot. 31: 289. 1898.

Measurement of photosynthetic action may be accomplished by the estimation of the amounts of carbon dioxide absorbed, and oxygen given off, or by the determination of the amount of the products of photosynthesis.

294. Demonstration of the Accumulated Product of Photosynthesis: Iodine Test. Place two plants of *Tropæolum* in the dark room for a day and then bring one of them into strong light in the morning. Near the close of the afternoon take a leaf from the illuminated specimen, and boil it for a minute in water. Place the boiled leaf in a beaker containing alcohol and warm to 50 or 60° C. until the green color is extracted. Prepare a saturated solution of chloral hydrate, and color it slightly by the addition of a solution of iodine. Pour some of this solution into a shallow glass tray, and put into it the bleached leaf, which has been rinsed in water. The chloral hydrate is a cleaning reagent and will render the leaf transparent, and the iodine will color the starch. The density of the coloring will denote the amount of starch present. If a leaf from the darkened plant is treated at the same time, the difference in the amounts of starch present may be seen at a glance, by the different color of the stained leaves.

295. Accurate Estimation of the Amount of Carbohydrates in Leaves in Darkness and in Light. Take all of the leaves from two plants treated as in the last experiment and find the total amount of carbohydrates in the one exposed to light, and in the one kept in darkness for 48 hours, according to the methods described in 223, 224 and 225.

296. Growth of Plants in Darkness, and in Air Lacking Carbon Dioxide. It has been found that plants grown in darkness are unable to absorb and make use of carbon dioxide since the supply of energy necessary to carry on photosynthesis is not furnished. If a green plant is compelled to live in an atmosphere lacking carbon dioxide it is unable to carry on photosynthesis, and pathological phenomena ensue which result in the disintegration of the chlorophyl and finally in the destruction of the plant. It will be of interest to compare the behavior of the

plant when cultivated without carbon dioxide, with that of others deprived of mineral salts.

297. Culture of Plants in Atmosphere Lacking Carbon Dioxide. A bell-jar with a tubulure at the top is necessary to carry out this experiment as described. The tubulure is closed with a
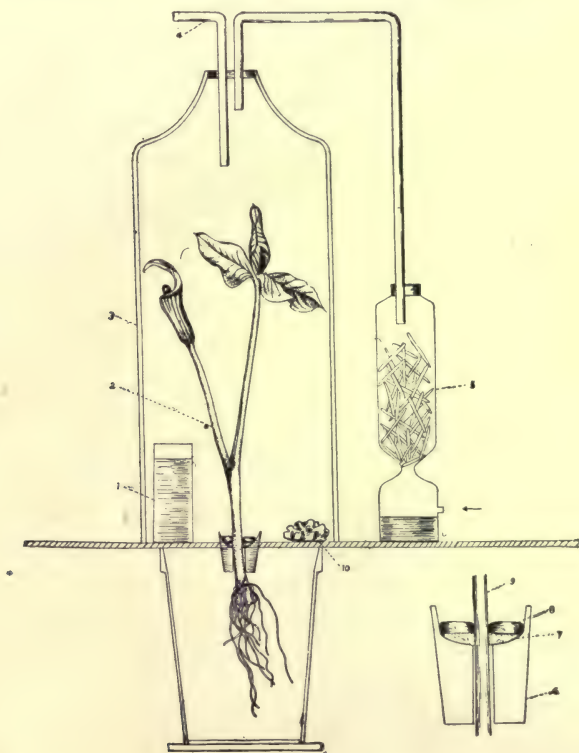
FIG. 113. Apparatus for growing plants in an atmosphere free from carbon dioxide. 1, dish containing solution of potassium hydrate. 2, specimen of *Arisaema triphyllum* ten days after opening of bud. 3, receiver of ten liters capacity. 4, outlet-tube connected with aspirator. 5, sticks of potassium hydrate and moist asbestos fiber. 6–9, details of method for sealing plant in receiver. 6, cork. 7, asbestos fiber. 8, mercury. 9, stem of plant. 10, sponge saturated with water.

stopper containing an outlet tube connecting with an aspirator or filter pump, and an inlet tube connected with a cylinder filled

with pumice stone, saturated with sodium hydrate absorbed from moist sticks of that substance mixed with it. A vessel containing a solution of potassium hydrate is also placed in the bell-jar together with a sponge containing water. Small plants cultivated

FIG. 114. *Arisaema triphyllum* grown in open air.

in pots may be set in a glass plate in a position receiving strong diffuse light and the bell-jar set over it. The edge of the jar should be sealed with vaseline, or wax, and all of the joints should be made air-tight. The plant will use all of the carbon dioxide in the jar not absorbed by the potassium hydrate in a few hours,

and will throw off an equal amount of oxygen, and the experiment may be carried out without renewing the air. In order to give more normal conditions, however, the pump is started so that a slowly moving stream of air is drawn through the material containing potassium hydrate, which removes all of the carbon dioxide and about all of the water at the same time. The evaporation from the sponge renews the moisture and makes an atmosphere of average humidity. At the same time the solution of potassium kept in the bell-jar absorbs the carbon dioxide given by the plant as a result of respiration. The ventilation of the bell-jar need not be carried on continuously. The pump may be allowed to run an hour or two in the morning, and the same period in the afternoon. It will be best to interpose a wash bottle containing a solution of potassium hydrate between the pump and the bell-jar to prevent any possible contamination by backward movement of unfiltered air. Replace this wash-bottle by one containing barium or calcium hydrate occasionally during the ventilation and note whether the liquid becomes milky because of the passage of the air from the bell-jar. If it does, the presence of carbon dioxide in the jar is proven and the experiment must be amended to exclude it by a more thorough filtering processes.

Observe the behavior of seedlings of *Phaseolus*, or *Zea*, under the conditions described. A certain amount of growth is carried on by the use of food stored in the seed but when the plant becomes dependent upon photosynthetic activity it perishes. Compare the behavior of seedlings with that of small bulbous plants. The shoots of large plants may be allowed to grow through a perforated glass plate and then covered with the bell-jar. The opening around the plant may be closed with a cork sealed with wax to the glass, and made tight around the plant by a seal of mercury and water (See Fig. 113). Ten to fifteen days will be necessary to reach conclusive results with most plants. Make a microscopical examination of the structure of the leaves after the effects of the lack of carbon dioxide have become visible, and compare with that of normal leaves.

It will be necessary to renew the material in the jar containing the pumice stone and potassium hydrate at least twice during the test.[1]   Test the composition of the atmosphere in the bell-jar at the close of the experiment by the method described in 302.

298. Conditions Affecting Photosynthesis.
It has been shown by the preceding experiments that the chief factors in the mechanism of photosynthesis are light, chlorophyl, and the presence of carbon dioxide in a gaseous form.   In addition it is to be said that a chloroplast is unable to carry on this process, until a certain stage of its formation has been reached, and also that if the chlorophyl in a plastid, is bleached beyond a certain point it may not be renewed and the chloroplast is destroyed.   The synthetic process may be continued in arctic species until the plant is actually frozen while in others it is inhibited by a temperature above the freezing point.   The maximum temperature is probably less than 50° C. in all species, and inhibition ensues at temperatures much below this in the greater majority of plants.   Both points are influenced by the amount of atmospheric moisture.

FIG. 115 *Arisaema triphyllum* grown in an atmosphere free from carbon dioxide.

Anaesthetics and all chemical agents which check the action of protoplasm, exercise a retarding and inhibitory influence on the process, while the accumulation of the products in the cells acts in the same manner.   It is probable that photosynthesis proceeds

[1] MacDougal.   Relation of the growth of leaves and the chlorophyl function. Jour. Linn. Soc. **31** : 526.   1896.

even in the feeblest illumination and that it ceases only in absolute darkness, while the increase of the illumination of some species to sixty times the normal did not accelerate the process. The intensity of illumination necessary to affect the process has not been accurately determined because of the difficulty in separating the effects of the visible rays from the heat effects.[1]

299. Influence of Amount of Carbon Dioxide upon the Amount of Photosynthesis. Secure some small leafy plant, or a shoot held in
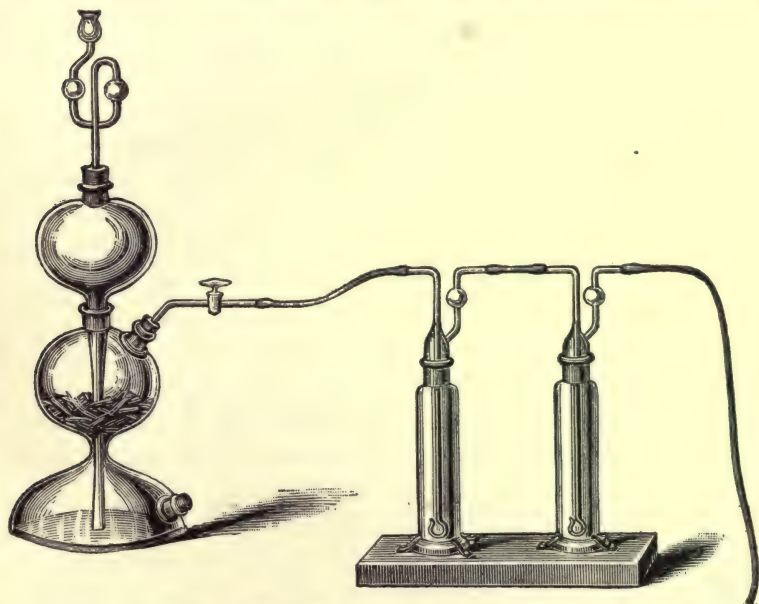
Fig. 116. Apparatus for generating carbon dioxide, connected with wash bottles.

a flask of water and place it in the tubulated bell-jar described in 302, Fig. 118. Connect a carbon dioxide generator in action and pass enough of this gas through a series of wash bottles and the capillary tube of the bell-jar to raise the proportion of the gas in the air of the bell-jar to 5 per cent. The burette on top of the bell-jar should be empty and open during the process. Close

[1] Ewart, A. J. On assimilatory inhibition in plants. Jour. Linn. Soc. **31** : 364. 1895-1897.

the connections, fill the burette with mercury, or water, and draw out a sample of air for analysis to determine exactly the proportion of carbon dioxide present. Allow the bell-jar to remain exposed to the light for three or four hours, then make a second estimation. What proportion of this gas has been used in photosynthesis? Repeat with 10 and 20 per cent. of the gas. What is the result in both instances?[1]

300. Influence of Temperatures upon Photosynthesis. Set up the experiment as in 301 using 10 per cent. of the gas and test the amount used during a period of three hours at a temperature of 20° C. Repeat and place a large piece of ice in the bell-jar, or use any method by which the temperature may be reduced to 12–15° C.
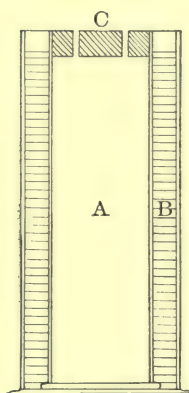
FIG. 117. Two cylinders arranged to expose plant to light which has passed through colored solutions. c, large cork, with perforations for tubing. b, layer of colored fluid.

301. Influence of Various Portions of the Spectrum upon Photosynthesis. Secure two tall cylinders of a height of about 30 cm., of a diameter of about 8 and 12 cm. respectively. Place a piece of lead or iron in the bottom of the smaller one and set it inside the larger. Fill the larger one with a liquid which will permit only red rays to pass. Place a shoot of any convenient plant inside the smaller cylinder and fit it with the apparatus attached to the bell-jar in 302. Run in enough carbon dioxide to make about five per cent. of the enclosed air, close the cylinder tightly by means of a large waxed cork stopper through which the various tubes pass, and set in sunlight for three hours. Make an estimation of the amount of carbon dioxide present at the beginning and end of the test. Repeat, using a solution in the outer cylinder which will permit

[1] See Schaible, F. Physiologische Experimente ueber das Wachstum und die Keimung einiger Pflanzen unter verminderten Luftdruck. Beitr. z. Wiss. Bot. **4** : 94. 1900.

only blue violet rays to pass, and determine how much carbon
dioxide is used.   Care must be taken to carry on the tests in the
same temperature and intensity of illumination.   It may be neces-
sary to use shades or shields to prevent unchanged light entering
the inner cylinder from the upper exposed part.   A thermometer
should be placed in the cylinder in such position that it may be
read during the course of the exposure.   The various solutions
will doubtless exhibit different diathermanic properties, and the
temperatures may be regulated by any convenient method (See
color filters 200).

302. Volumetric Estimation of Atmospheric Gases.   The follow-
ing method will be found suitable for estimation of atmospheric
gases in all tests of exchanges between the plant and the air, both
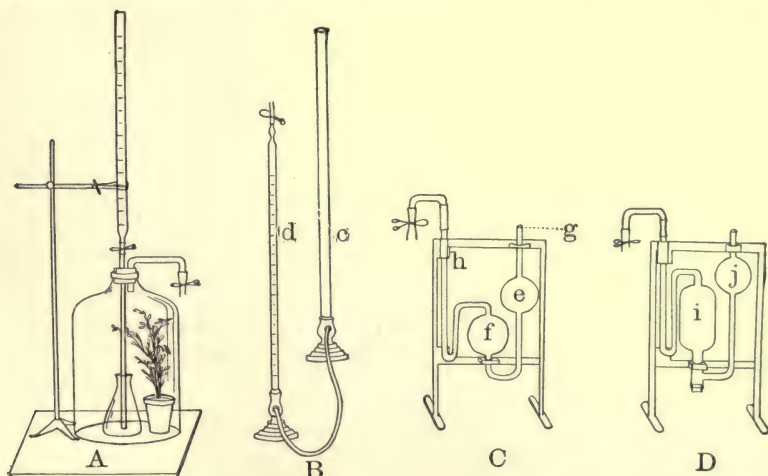
Fig. 118. Apparatus for estimation of atmospheric gases.   *A*, bell-jar covering
plant, with capillary tube closed by rubber tube and clamp, and burette with stop-
cock.   *B*, Hempel's gas burettes.   *C*, gas pipette containing solution of potassium
hydrate.   *D*, gas pipette containing sticks of phosphorus.

in photosynthesis and respiration, and also commends itself from
the fact that the apparatus consists of standard chemical burettes,
etc., kept in stock everywhere.

Place the experimental plants on a suitable ground-glass plate

and place alongside a small empty flask. Smear the edges of a bell-jar provided with a tubulure at the top with a cerate consisting of equal parts of tallow, beeswax and linseed oil and cover the plant and flask, taking care to seal the jar tightly to the plate. Provide a tightly fitting rubber stopper with two holes for the tubulure. A glass tube extending to the bottom of the flask and projecting a few centimeters outside the stopper is inserted in one opening and is connected with a burette, suitably supported, by a short section of rubber tubing clamped by a pinchcock, or a burette with a stopcock may be used. Insert a small section of glass tubing with capillary bore bent twice at right angles in the other opening of the stopper. This tube should be closed with a section of rubber tubing and a pinchcock (Fig. 118, A).

After the desired length of time has elapsed and it is necessary to test the proportion of oxygen and carbon dioxide in the jar, fill the burette attached to the tube with mercury. Next provide a pair of Hempel's gas burettes (Fig. 118, B). Fill slightly more than half full with water. Raise the open burette c until the graduated burette d is filled with water. Connect the rubber tube with which the upper end of this burette is closed with the capillary tube leading into the bell-jar and open the stopcocks ; now lower the other burette until the level of the water in the graduated burette is half way down its length, and about 50 cc. of air have been withdrawn for analysis. Bring the fluid to the same level in both burettes and measure exact amount of air in closed burette which will be at normal pressure. Now allow the same amount of fluid to flow into the bell-jar from the burette above it to equalize the tension.

Fill the absorbing pipette (Fig. 118, C) with a solution consisting of 1 part potassium hydrate and 2 parts water until the liquid rises a little into e. Force air in at g until the potassium solution is forced up and fills the tube to the stopcock. Connect with the burette containing the air to be tested and open the stopcock and manipulate the pair of burettes to allow the air to be drawn into the absorption pipette where it remains 3 to

5 minutes, the potassium solution absorbing the carbon dioxide. Lower the open burette *c* and draw the air off into the calibrated burette and read its volume. The difference between this and the previous reading will denote the amount of carbon dioxide originally present (See Fig. 119).

Fill the cylindrical bulb of a second absorption pipette with sticks of phosphorus about 3 mm. in diameter and fill with distilled water. Draw the air in the graduated burette into this pipette and allow it to remain for a few minutes and the oxygen will be taken up by the phosphorus. Force back into the burette and measure as before.

It is important that all connections should be made with tubing of a capillary bore and care must be taken throughout that no air except that taken from the bell-jar is included in the portions tested (See also 327).

FIG. 119. Hempel's gas burettes attached to pipette containing potassium hydrate, showing method of forcing air into the latter.

303. Photosynthesis by Bacteria. A number of bacterial forms are found to give off oxygen when illuminated. Certain of these including *Chromatium Okeni* are furnished with chlorophyl and carry on photosynthesis. Others, however, furnished with some compound of lipochrome, hold oxygen in loose combination and give it off when exposed to light.[1]

304. Chemosynthesis of Carbohydrates. The synthesis of carbohydrates by means of energy derived from chemical compounds has been demonstrated in the nitrate and nitrite bacteria only. The nitrite bacteria absorb and oxidize ammonia to nitrous acid, and

[1] Ewart, A. J. On the evolution of oxygen from colored bacteria. Jour. Linn. Soc. **33 : 123.** 1897.

by means of the energy derived from this process are able to use carbon dioxide of the air or that which has been in combination with the ammonia in the construction of the carbohydrates. The nitrate bacteria oxidize nitrous acid, and obtain energy from which similar synthetic processes are made possible.[1]

305. Chemosynthesis of Nitrogenous Substances. Before the carbohydrates formed by photosynthesis may be assimilated by living substance they must be formed into new compounds containing nitrogen, in such manner as to constitute a proteid. This combination is made between glucose, or maltose, on one hand and nitrates, or ammonia on the other. The presence of sulphates and phosphates is also necessary, and the acids named are generally in the form of salts of magnesium and potassium. Calcium does not appear to take any direct part in the process, yet it is necessary to neutralize injurious bye-products. The synthesis of the proteids appears to take place most rapidly in cells containing chloroplasts, in light, probably because of the greater abundance of the carbohydrate, although it may occur in any part of the plant and is therefore a chemosynthetic process. No other source of energy is available to fungi by which it can be carried on. The nature of the first product of the synthesis has not been determined, although supposed by several investigators to be amides. In such instances the end reaction might be expressed by the following formula :
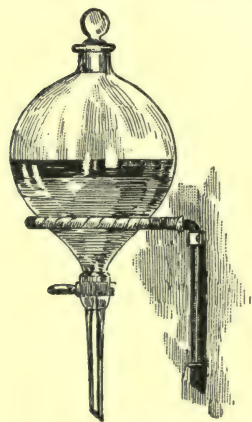
FIG. 120. Service vessel for mercury consisting of a separatory funnel of a capacity of a liter, supported by bent rod, with curved end.

$$C_6H_{12}O_6 \ + \ 2KNO_3 \ = \ C_4H_8N_2O_3 \ + \ K_2C_2O_4 \ + \ H_2O \ + \ O_{30}.$$

Glucose + potassium = asparagin + potassium + water + oxy-
nitrate   oxalate   gen-
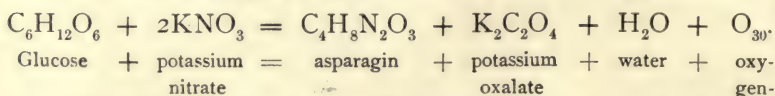
The amide, asparagin, formed is diffusible and might be easily

[1] See literature list, Pfeffer. Plant Physiology. 1 : 361. 1900.

translocated. The calcium present would form insoluble crystals of oxalate which are always found after the synthesis of proteids. The relative share of the nucleus and cytoplasm in the synthesis have not been ascertained.[1]

306. Translocation of Plastic Material. Substances such as the carbohydrates, formed, or stored, in some special organ of the plant are often conveyed long distances through the body to tissues where they are used for various purposes. The carbohydrates in the leaf are transported to the root, and the material accumulated in underground storage organs and seeds is carried to the newly developed shoots and branches. In all cases the material to be moved is converted into some form readily diffusible. Carbohydrates are converted into glucose or maltose, although some translocation may occur in other forms, and proteids are hydrolyzed, or broken up into amides, and again reformed into albuminous bodies upon reaching the tissue to which they were attracted. The transportation of food, or plastic material from one organ to another is largely a matter of osmotic attraction, although the physical process is under the control of the protoplasts, which have the power of varying the permeability of the plasmatic membranes from time to time. The use of any plastic substance, or its conversion into an insoluble form in any cell, reduces the concentration of the solution in the cell in question, and a supply flows in to equalize the osmotic balance, and in this way a stream of carbohydrate may flow from the leaf to the root. The conversion of insoluble or indiffusible into soluble diffusible form is generally effected by the action of enzymes (See Enzymes). Carbohydrates are formed in the leaves about ten times as rapidly as they may be removed by translocation in ordinary species. During the period of illumination the amount which might not be diffused from the cell in which it was formed is condensed into starch by the action of pyrenoids, or certain

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[1] Hanstein, B.   Ueber Eiweisssynthese in grüner Phanerogamen.   Jahrb. Wiss. Bot. **33** : **417.**   1899.

Schulze, E.   Ueber Eiweisszerfall und Eiweissbildung in der Pflanze.   Ber. Deut. Bot. Ges. **18** : 36.   1900.

centers of activity in the chloroplasts, or by independent leuco-plasts. During the night the diastase found in the plastids hydrolyzes the starch, converting it into glucose or maltose, which then may pass out of the cell to be again condensed a number of times in its way to the root, or deposited in a storage tissue by the action of other plastids (leucoplasts). Other carbo-hydrates, such as reserve cellulose, may be acted upon similarly. The proteids are subjected to the action of peptonizing ferments which convert them into soluble form and enable them to diffuse as described above.

307. Channels for the Conduction of Plastic Material. The transportation of material from the point at which it is absorbed, or formed, to the tissues in which it is used, or stored, is accom-plished in a variety of ways. The mineral constituents taken up by the roots pass through the root hairs and cortex into the xylem of the roots, and then pass upward through dead cells dif-fusing laterally by osmotic attraction to the embryonic and cortical tissues. The movement of carbohydrates and other complex bodies takes place most rapidly through the sieve cells, and other elongated elements in the phloem, also diffusing laterally into the cortex. In trees the lateral movement is made largely through the medullary rays. Elongated cells with a comparatively small number of septae facilitate the process. In the lower plants with undifferentiated tissues, conduction must be accomplished by osmosis through cells of small diameter, and is sufficient here to move material through the distances intervening between the dif-ferent parts of the body. The streaming movements of proto-plasm doubtless aid in the process, although such movements are not sufficiently prevalent to be considered as a general factor in the process. Involuntary movements of the liquids in cells due to bending and twisting from the force of the wind, or water, must also be of benefit in translocation. Latex and resin are present in quantity in many plants and are capable of transportation through systems of tubes and canals continuous without partition walls throughout the body of the plant.

If at any time a substance in translocation accumulates in a conducting tissue, it may be reconstructed, or condensed into some insoluble form. Starch which is thus formed is known as transitory starch. Such accumulations of starch are not to be taken as marking special conducting tissues however, since the accumulation may occur in tissues lateral to the main conducting elements, such as the bundle sheath in which the longitudinal translocation is comparatively slight.

Movements of sap through dead cells is not affected by anaesthetics, but is decreased by mechanical compression. On the other hand anaesthetics and lack of oxygen stop the movement of material in translocation through living cells, due probably to alterations induced in the plasmatic membranes.

308. Translocation of Carbohydrates from Leaves. Expose some plants of *Solanum* or *Cucurbita* to strong illumination, under favorable conditions, during an entire day. In the evening test some of the leaves for starch, which should be found plentifully. Cut off a few of the leaves and put in a moist chamber. On the following morning test some leaves taken directly from the plant and those which have lain in the dark chamber for starch. Those attached to the stem will be found to have been emptied of starch, while about the original amount is present in the detached leaves. The test may be made still more striking and conclusive if the leaves are tested for the total amount of carbohydrates present, by the methods described in 220–226. Another interesting example of translocation is offered by germinating seeds. If the contents of the cells of a resting bean are examined, and compared with those of a seedling which has developed the first pair of leaves, it may be seen that a large amount of the food material stored in the seed has been transported or withdrawn, and presumably used in constructing the growing shoot and root-system.

309. Storage of Reserve Food. Material which might serve as foods is formed much more rapidly than it is used in furnishing energy to the plant, or building material for morphological construction. This tendency to accumulate potential in the shape

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of chemical compounds is characteristic of the greater number of vegetal organisms. Surplus material is conducted away from the point of formation, and generally deposited in the tissues in some form not readily diffusible. Such deposition may be made in spores, thalli, roots, stems, branches, leaves, floral organs, seeds and fruits, etc. The general purpose of such accumulation is to afford nutriment to the growing cells in the succeeding vegetative period. A great many instances might be cited however, in which the food-material placed in a fruit actually serves only to attract animals which consume it, and carry the seeds to other possible habitats. Again reserve materials may be poisonous to animals and thus serve no other purpose than that of general protection.

Starch is the most abundant and widely distributed reserve substance. It may be formed by the action of the chloroplasts or by other plastids (leucoplasts) in various parts of the body, and is an extremely economical substance for storage purposes. It is not formed by the fungi, although the plastids of chlorophylless seed plants are capable of constructing it from other carbohydrates. Glycogen, a carbohydrate closely related to starch, is formed in the fungi, and is generally in solution in the cell-sap although sometimes deposited in amorphous form. Inulin is a reserve carbohydrate found in Compositae, Liliaceae, Amaryllidaceae and other Monocotyledons. Cane sugar is used as a reserve food in the sugar beet, and sugar cane, and grape sugar is stored in many forms. Layers of reserve cellulose are deposited on the walls of cells, especially in seeds, of which the common date of commerce affords a good example. Proteids are stored in the form of aleurone grains, and in crystals. Gluten occurs in the seeds of certain grasses. Amides, such as asparagin, are to be found in the sap of many plants, although in most instances it is simply the transitory form of the more complex proteids. Glucosides are particularly abundant in the Cruciferae and allied orders. Fats and oils are abundant in seeds and also are often found in fleshy roots. These substances, like starch, are gener-

allo formed as the result of the activity of special plastids termed elaioplasts. Crystals of various mineral salts, and the varied contents of laticiferous tissue including resin, also may be included among the reserve materials, and even the poisonous acids and alkaloids may sometimes be regarded as serving similar purposes.[1]

310. Determination of the Storage Substances in a Plant. Secure several specimens of *Helianthus, Solanum, Avena, Pisum* or *Phaseolus* and make a complete examination to identify and locate the different substances stored as reserve food in various organs.

311. Formation of Storage Organs and Deposition of Reserve Material. Cultivate a number of specimens of *Solanum* from cuttings of tubers, and follow the development of the new storage tuber formed at the base of the new stems. Note the enlargement of the tissues and the accumulation of the reserve material (See formative effect of light).

312. Special Types of Nutrition. The method of nutrition by which mineral salts in simple combinations are taken up from the substratum, and carbon dioxide is absorbed from the air is the prevailing one in the vegetable kingdom. The essential feature of this method is the absorption of energy direct from solar radiations by means of a specially developed chlorophyl screen. Practically all organic substances have been constructed by means of the energy thus derived. Plants as a group build up many times as much material as they use in growth and development, and the death of the successive generations of individuals adds to the store of organic matter on the surface of the earth, which is constantly undergoing decay and decomposition, forming humus in the process. The remains of plants contain all of the substances formed in living material, but in various stages of disintegration into simpler compounds. The compounds in the humus contain much more chemical energy than the simple salts forming the major portion of the soil products used by green plants, but their complexity is such that they do not easily pass the plasmatic

[1] Pfeffer. Physiology of Plants, **1** : 604. 1900.

membranes of absorbing organs.   It is definitely established how-
ever that all green plants take up minute proportions of such
organic food, and to that extent lessen their need for the products
of photosynthesis.   A large number of plants including the great
groups of bacteria (except a few forms, 303, 304) and fungi have
lost the power of forming chlorophyl, and of photosynthesis, and
obtain their food from substances absorbed from living or dead
organisms.   This variation is shared to some extent by seed
plants also.   Species which derive their food from decaying organic
matter are termed saprophytes.[1]   Only one species of seed plant
is supposed to be able to live wholly in this manner, although this
point needs further investigation.   A large number exhibit various
degrees of saprophytism however, among which are to be re-
counted the carnivorous forms which receive or entrap animals, the
decaying remains of which are used by the plant.

Many species attach themselves to the bodies of other organ-
isms, and derive all of their food-supply from their host, or only a
part of it, being furnished with a modicum of chlorophyl, and
hence able to carry on some photosynthesis.

In another general type of nutrition two or more species as-
sociate together in such manner that an exchange of material en-
sues between them, resulting in various degress of benefit to the
members of the partnership.   Such associations constitute a sym-
biosis.   One form of such symbiosis, in which saprophytic fungi
are associated with the underground organs of various pterido-
phytes, gymnosperms and phanerogams, constituting mycorhizas,
is very widely prevalent.   Perhaps the greater number of all the
higher plants enter into such combinations, and receive a small
proportion of their total food-supply by exchange with the fungi
attached to their underground organs.   Certain fungi and algae
associate in this manner to form the lichens, a distinct group in
the vegetable kingdom.

313. Nutrition of a Saprophyte.   The following test will demon-
strate the substances used by a saprophytic fungus.   Soak a slice

[1] MacDougal,. Symbiosis and saprophytism. Cont. N. Y. Botanical Garden.   No.
1. 1899.   (Rep. Bull. Torr. Bot. Club. 1899.)

of bread in water for half a day, then place it in a dish under a
bell-jar in a room at ordinary temperatures. A number of
species of whitish moulds will be produced at first, followed in a
few days by masses of bluish *Penicillium*.   Both kinds live on the
organic material contained in the bread, but the determination of
the food constituents may be made by the following cultures.
Make a nutrient solution as follows :

  100 cc. distilled water.

  .05 gram ammonium phosphate.

  .05 gram acid potassium phosphate.

  .03 gram magnesium sulphate.

  .01 gram calcium chloride.

  A drop or two of iron sulphate.

Divide the solution into four parts and place in Petri dishes.
Leave one dish unchanged, to a second add .02 g. grape sugar, to
the third add .02 cc. oxalic acid, and to
the fourth add .02 cc. citric acid. Acidu-
late the first and second dishes with a
drop or two of dilute sulphuric acid.
Take a small mass of spores of *Penicil-
lium* from the bread culture and place
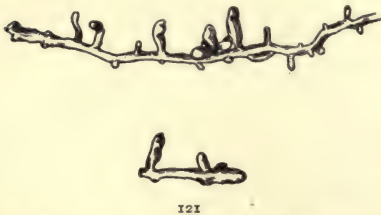
FIG. 121. Portions of root of *Tsuga Canadensis*, with club-shaped mycorhizas.
After Harlow.
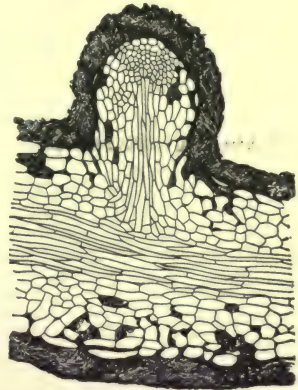
FIG. 122. Longitudinal section of mycorhizal root of *Tsuga Canadensis*. The
outer layers are inhabited by a fungus. After Harlow.

in each dish. Set the dishes in a dark room at a temperature
of 16–20° C. and note condition a week later. This should
demonstrate which of the added organic substances will serve as

food for *Penicillium*, and that the solution of mineral salts alone is not sufficient.   Many other organic substances may be tested in this same manner.

314. Mycorhizas: Associations of Higher Plants and Saprophytic Fungi.   Take up a mass of the finer roots of beech, oak, or any coniferous tree and carefully wash away the adherent soil.   Note the club-shaped branches of the smaller roots, constituting mycorhizas.   Cut cross and longitudinal sections of the structures, and note the position and development of the fungus which may form a layer of hyphae around the root replacing the root hairs.   The fungi may occupy the external layers of the root, or may live in the cortical tissues sending branches of the hyphae out through root hairs in other instances.   Make microchemical tests, and ascertain the nature of bodies found in the hyphae, or their enlargements, and the substances in the bodies of the higher plants used as food by the fungi.   The types which will come under observation in this manner are examples in which the higher plant receives only a small proportion of its nourishment from the associated fungus.   Many seed plants have developed this habit so strongly that they receive almost all of their food material from the fungus, and carry on transformations with the material received, of which the lower plant is incapable, and yield the product to the fungus.   It will be profitable to examine two examples of this type, of which *Monotropa* has entirely lost its chlorophyl, and *Corallorhiza* which retains a small amount, and is presumably able to carry on more or less photosynthesis.

FIG. 123. *Corallorhiza odontorrhiza* with coralloid mycorhizas formed from subterranean branches.

Obtain clumps of *Monotropa* from the woods in the autumn and carefully separate the roots from the adherent humus.   Cut sections and ascertain the anatomical relations of the two plants.

Note the degeneration of the shoot of *Monotropa* as a result of its altered nutritive relations (Fig. 125).[1]

Secure clumps of any species of *Corallorhiza* and wash away the soil. The coralloid underground organs are found to show inter-
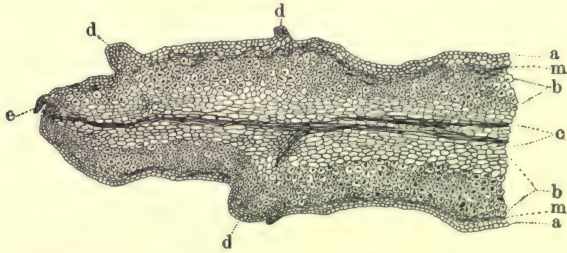
FIG. 124. Longitudinal section of apical portion of mycorhiza of *Corallorrhiza Arizonica*. *a, a,* epidermis. *m, m,* mycelial layer of fungus. *b, b,* cortical region of branch in which organs of interchange of the fungus are formed. *c,* stele. *d, d, d,* secondary branches. *e,* scale-leaf at apex of branch.

nodes, and hence are stems, the roots having been lost as a result of the mycorhizal adaptation. Cut cross and longitudinal sections of some of the smaller branches. Note the tubular extensions of
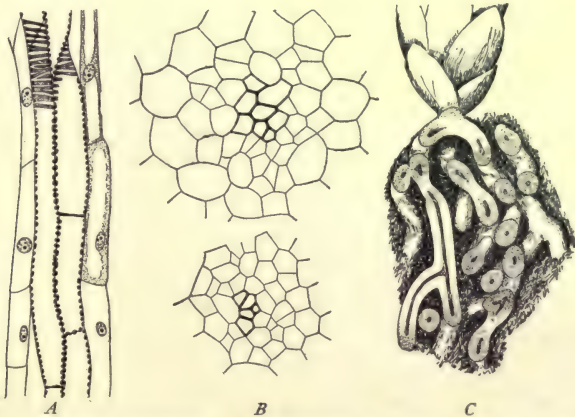
FIG. 125. *Monotropa uniflora*. *A,* longitudinal section of stele of root showing vessels and contiguous phloem cells. *B,* transverse sections through mature and young roots showing vessels. *C,* mass of roots from which arises a flowering shoot.

[1] MacDougal and Lloyd. The roots and mycorhizas of some of the Monotropaceae. Bull. N. Y. Bot. Garden. **1** : No. 5. 419. 1900.

the epidermal cells resembling root hairs. The fungus will be found to penetrate deeply into the cortex, forming dense clumps in the interior cells, and looser coils in the outer layers. The hyphae in the outer layers may be regarded as the mycelium of the fungus which grows forward as the branch extends, sending internal branches into the cortex and external branches out through the trichomes into the humus. Material absorbed by the external hyphae is brought into the mycelium and its internal branches, undergoing more or less synthetic change, and some it diffuses into the protoplasm of the higher plant. Ascertain what material in the cells of the *Corallorhiza* is taken up by the fungus, and the probable nature of the material obtained by *Corallorhiza* (Fig. 124).[1]

315. Arrangement of Components of Lichens. Examine the structure of a *Cladonia* or *Sticta* and ascertain the structural relations of the fungus and alga associated.[2]

316. Relations of a Fungous Parasite to its Host. Secure plants of *Bursa Bursa-pastoris* (*L.*) Britton (*Capsella Bursa-pastoris*) showing the whitish pustules due to *Albugo candida* on the stems, leaves, flowers and seeds. Cut sections of the material and stain in a solution of potassium iodide and iodine. Note the relations of the parasite and its host. Find the special absorbing organs, the haustoria of the parasite. Some of the facts may be brought to light more easily, if a fragment of the infested material is boiled for a minute in a solution of potassium hydrate, washed with distilled water, then stained with the iodine solution.

The structural arrangement of a parasite may also be seen by an examination of the aecidium which is parasitic on *Peltandra* or *Arisaema* showing yellowish pustules on the surfaces of the leaves.

[1] MacDougal. The significance of mycorhizas. Biological lectures, Woods Holl Marine Laboratory, p. 49. 1899.

Stahl, E. Der Sinn der Mycorhizenbildung. Jahrb. Wiss. Bot. **34** : **539.** **1900.**

Magnus, W. Studien an der endotrophen Mycorrhiza von *Neottia Nidus avis* L. Jahrb. Wiss. Bot. **35** : Hft. 2. **1900.** Sarauw, G. F. L. Rodsymbiose og Mykorrhizer saerlig hos Skovtrœerne. Bot. Tiddsskrift. **18** : **127.** **1893.**

[2] Schneider, A. Text-book of general lichenology. **1897.**

317. Relations of a Phanerogamous Parasite and its Host. Secure a number of seeds of *Cuscuta* in the autumn and keep in a cool place until needed in the experiment room. Germinate a number of seeds of *Helianthus*, or *Impatiens*, in a pot filled with soil and when the shoots have reached a few cm. in height sow the seeds of *Cuscuta* in the soil around the plants. Note the behavior of the seedlings of *Cuscuta*. After a time the parasite will coil around the host plant and attach itself by means of special outgrowths, the *haustoria*. Examine the structure of these. Cut cross sections of the stem of the host at points penetrated by the haustoria and note their action. The anatomical relations of the two plants may be seen if material is taken in August and preserved in alcohol, or formalin until needed, although this plant offers an easy demonstration of the stimuli serving to direct the parasite in its attachment to a host. Test the stem of *Cuscuta* for chlorophyl (184).[1] Examine also plants of *Epiphegus*, *Phoradendron*, *Arceuthobium*, or any convenient parasite and note the effects of the parasitism on host and parasite.

[1] Peirce, G. A contribution to the physiology of *Cuscuta*. Annals of Botany, **8**: 53. 1894.

Mirande. Recherches physiologiques et anatomiques sur les Cuscutacées. Bull. d. Sc. d. France et d. l. Belge. **25**: 1900.

XII. RESPIRATION, FERMENTATION AND DIGESTION

318. Derivation and Conversions of Energy. Solar radiations constitute the ultimate source of all energy in the organic world. The waves of light act only upon the external layers of the body of a plant and with intermittent periods of darkness. The activity of protoplasm is almost continuous however, so that it has become necessary for it to absorb energy from light during periods of illumination, and store it up for use when needed. In order to accomplish this the kinetic energy of light is converted into potential in the complex chemical compounds formed in photosynthesis, and these may be translocated to any part of the body and stored for indefinite periods, and it has been pointed out in previous sections of this book that the amount of energy accumulated in this manner is generally much greater than that used by the individual plant itself.

Any other organism such as an animal, or another plant that can assimilate these compounds without previous disintegration, may acquire and use their contained potential. This is accomplished by most bacteria, fungi and chlorophylless seed plants. It is also possible that some forms acquire energy from heat radiations.

Material built up in this manner may be used in construction with no disintegration or liberation of energy, or it may be broken up to obtain the energy which it contains in potential form. Thus with a given amount of wood some of it may be used to form the timbers of a house or bridge, while the remainder is burned in an engine, to obtain energy to cut the boards and hoist them into position.

The evaporation of water in transpiration, and the accompanying physical processes use more than 98 per cent. of the energy absorbed from sunlight by a plant, and all of the other work of the organism is accomplished by means of the remaining 2 per

cent. This is converted and stored as potential energy in photo-synthesis, which is a reducing process, oxygen being set free. The material thus formed is carried to all parts of the body and furnishes energy for growth, morphological construction, move-ment, and maintenance of the rigidity and position of the body.

Translocations of the compounds allows energy to be liberated in the particular cells in which it is needed. During the libera-tion of potential energy by physiological combustion some of it is converted into kinetic forms such as heat, which is but of little use to the plant and so is lost.

Two principal types of liberation of energy may be designated as aërobic and anaërobic respiration. Aërobic respiration con-sists in the oxidation of the complex compounds of living matter, or of the substances which saturate the meshwork, in a manner which if completely carried out results in the formation of water and carbon dioxide. The combustion may proceed only so far as to produce organic acids however, and may not be accom-panied by any excretion or formation of carbon dioxide.

Anaërobic respiration (often termed intramolecular respiration) is the process by which disintegration and liberation of energy, in compounds in the cell, are produced without the aid of oxygen. Sugar and proteids may be broken up in anaërobic respiration producing carbon dioxide, water, sometimes hydrogen, nitrogen, ammonia, amido-acids, and most generally alcohols. In one form of anaërobic respiration special substances known as enzymes are secreted by protoplasm which produce decomposition of various compounds by fermentative action, or such action may be exercised directly upon compounds in its meshwork (See oxidases). It is to be noted however, that not all fermentative processes are respiratory, since some of them are purely digestive in their purpose. All of the above methods of liberation of energy may proceed side by side, and the various steps in the separate processes are not well known.

Respiration goes on continuously in all organisms, but is re-duced to a minimum in living plants in a desiccated condition,

such as dried mosses, lichens and seeds in which it is practically zero. Absolute cessation of respiration should occur in seeds and bacteria exposed to the extreme low temperatures of liquid hydrogen (— 252° C.).

319. Aerobes. A supply of free oxygen is necessary to the continued respiration and existence of aërobes, although such forms are capable of liberating sufficient energy for shorter periods by means of anaërobic processes. The external manifestations of aërobic respiration are the excretion of carbon dioxide (not always shown), heat, which may be diffused so rapidly as to be incapable of measurement, and in rare instances, phosphorescence

Since physiological combustion is not always complete, it is evident that the proportion of carbon dioxide to the amount of oxygen used, must vary greatly. The respiration of oily seeds produces less of this substance than the amount of oxygen absorbed, but in seeds containing starch or sugar the amounts are practically equal, while *Penicillium* is claimed to excrete 2.9 times as much carbon dioxide as oxygen absorbed when fed on tartaric acid, although this disproportion is doubted by some writers. Respiration is most rapid in the more vigorous parts of the plant, although not always in the regions showing the most rapid growth, and the amount of carbon dioxide excreted may amount to six per cent. of the bulk in a mould, and as much as 2.4 per cent. of the bulk of the organism, daily in certain bacteria. A temperature of — 10 to — 15° C. is probably the minimum for respiration, and the optimum probably lies at the maximum, or at the point of heat rigor. Light has but little influence on the process.[1]

Anaesthetics and narcotics may increase respiration, although their final and continued influence would lower the activity of the organism in several ways.[2]

[1] Puriewitsch, K. Physiologische Untersuchungen über Pflanzenathmung. Jahrb. Wiss. Bot. **35** : 573. 1900.

See also Palladine, M. W. Influence des changements de temperature sur la respiration des plantes. Rev. Gen. d Bot. **11** : 241. 1899.

[2] Morkowine, M. N. Recherches sur l'influence des anesthesiques sur la respiration des plantes. Rev. Gen. d. Bot. **11** : 341. 1899.

Injuries and wounds also tend to increase re-
spiration locally as shown by excretion of carbon
dioxide.[1]

Respiration is also influenced to a slight extent
by the pressure of the oxygen in the atmosphere.
Any decrease below the normal exercises a corre-
sponding effect on the excretion of carbon dioxide,
and stimulates intramolecular respiration, while an
increase above the normal accelerates the process
but slightly and is injurious to the organism. An
accumulation of the products of respiration retards
the process.

The evolution of heat is exhibited in a marked
degree by unfolding flowers, germinating seeds and
growing sporophores of fleshy fungi, and a tempera-
ture of many degrees above that of the surrounding
air may be reached. Phosphorescence as a result
of respiration is exhibited by a few special forms of
fungi.

**320. Demonstration of Excretion of Carbon Dioxide
During Aerobic Respiration.** Place a few dozen
seeds in a germinator until the roots are a centi-
meter long and then put into a glass cylinder of a
capacity of about a liter. Close the jar with a
ground-glass plate or stopper and keep at room
temperature. A day later cautiously push the
ground-glass to one side and thrust in the jar a
short piece of burning candle fastened to the end
of a wire. It will be quickly extinguished, de-
noting the absence of oxygen. Close the jar
and prepare a fresh solution of barium hydrate
in a closed test-tube. Secure two wide-mouthed

FIG. 126. Dem-
onstration of the
excretion of car-
bon dioxide in
germinating seeds.
A, mercury, which
is seen to have
risen in the tube at
the close of an ex-
periment. *B*, ger-
minating seeds. *C*,
support and bottle
of potassium or
sodium solution.
D, rubber stopper.

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[1] Zaleski, W. Zur Aetherwirkung auf die Stoffumwandlung
in den Pflanzen. Ber. Deut. Bot. Ges. **18** : 292. 1900.

Richards, H. M. Respiration of wounded plants. Annals
of Botany, **10** : 531. 1896.

bottles holding about 50 cc. Set one near the jar and pour a small amount of the barium solution into it, allowing the liquid to fall several centimeters in a thin stream from the test-tube. The liquid will be only slightly milky when it is shaken up in the bottle. Now carefully lower the second bottle by means of a cord into the jar and allow it to rest on the seeds. Hold the test-tube at the same distance as before and pour the remainder of the liquid into the bottle in the jar. After a few minutes take it out and shake, noting that it shows the liquid in a much more milky condition than in the first bottle. The two tests show that germinating seeds absorb the oxygen of the air, so that combustion is not supported, and that the air confined with the germinating seeds contains a larger proportion of the gas (carbon dioxide) which gives the barium solution a milky appearance, due to the formation of insoluble barium carbonate.

321. Ready Method of Estimation of the Amount of Carbon Dioxide Exhaled. Place about a hundred germinating seeds of wheat, or a number of opening flower buds, or a number of mushrooms in a state of rapid growth, in an Erlenmeyer flask on a layer of crumpled filter paper. Set one or two small test-tubes half full of saturated solution of sodium hydrate on the seeds, and then close the flask with a rubber stopper perforated to admit a short section of glass tubing bent at right angles twice. Support the flask on a retort stand and connect the free end of the bent tube with a graduated burette by means of a stout piece of rubber tubing wired and make all joints air-tight. Bring a dish full of mercury under the lower end of the burette, and warm it at the middle until a bubble of air escapes, and the mercury in the burette rises to the level of that in the dish on cooling. Mark the exact level. Set the apparatus in a place where it will not be exposed to direct light and note the temperature. The volume of the air in the flask will be decreased by the amount of oxygen absorbed by the seeds, which should be replaced by carbon dioxide excreted. This substance is absorbed by the sodium hydrate, however, as fast as it is formed, so the replacement is effected by

the rise of mercury in the tube. Note the amount of mercury drawn up 4 and 8 hours later. This experiment is of value only with plants in which the amounts of carbon dioxide exhaled and oxygen absorbed are equal. It is subject to the following errors : The proportion of oxygen in the air about the seeds is constantly decreasing, which would lessen respiration, and the downward pull of the column of mercury varying with the barometric pressure would also tend to make the result less than the normal. The actual volume of carbon dioxide given off will also be greater than that of the mercury drawn up in the tube.

This demonstration may also be accomplished in the following manner : secure a funnel tube with a cylindrical top with a capacity of 100 to 200 cc., and place 20 or 30 germinating seeds on a piece of filter paper in the cylinder. Bend a section of wire a few cm. in length to form a tripod support for a small bottle containing a solution of sodium or potassium hydrate. Close the upper end of the cylinder tightly with a large rubber stopper. Now support the funnel tube in a perpendicular position in a small dish of mercury. Warm the tube until some air is driven out allowing the mercury to rise to the same level in the tube and dish when cool. The volume of mercury drawn up in the tube represents amount of carbon dioxide absorbed, with corrections as above (Fig. 126).

322. Incomplete Combustion in Oily Seeds. A smaller proportion of carbon dioxide is given off in the respiration of oily, than starchy seeds. Soak a few dozen seeds of hemp for an hour, and then lay on moist filter paper in the flask used in the last experiment and set up as before, omitting the potassium. Note the slight rise of the mercury due to the fact that in the earlier stages of germination of such oily seeds, less carbon dioxide is excreted than oxygen is absorbed. Later however, the formation of starch restores the normal ration in the interchange of the two gases, and the rise of the column of mercury will soon cease.

323. Excretion of Carbon Dioxide in the Anaerobic Respiration of an Aerobe. *Pisum* is an aërobic species but the seeds are capa-

ble of carrying on an anaërobic respiration for extended periods, in which the amount of carbon dioxide given off is nearly that of the normal. The following test may be made. Soak six peas for twelve hours and then remove the coats without injury to the plantlets. Fill a calibrated test-tube with clean mercury, and support in an inverted position in a dish of mercury. Pass the peeled peas under the rim of the test-tube, using forceps to handle them, and do not allow air to gain access to the tube. After the peas have collected at the upper end of the tube pass in also a ball of filter paper about the size of a pea saturated with water. If any air has been allowed to gain entrance to the tube, it must be taken down and the operations described repeated. Observe 12 and 24 hours later. Note the amount of gas collected in the upper end of the tube. Its composition may be roughly demonstrated if a small stick of potassium hydrate is moistened in water and then passed up into the tube. If it is carbon dioxide the gas will be absorbed and the mercury will rise to nearly its former height.

324. Decrease of Dry Weight by Respiration. Select 30 good seeds of *Zea*, and determine the amount of dry matter in 10 of them (238). Place the remainder in a suitable germinator in a dark chamber, and when the roots are a few mm. long, bring 10 into the light and place in a water culture apparatus (292). A week later estimate the amount of dry matter in the lot growing in the dark room, and in those in the light. Compare the average weight per seed in the three lots. The dry weight should be slightly decreased during germination, but this loss would be compensated by the synthesis of carbohydrates in the specimens brought into the light. The etiolated plants should show a continued loss. It is important that the material should be handled carefully and that the remains of the seed attached should be included in the analyses. On this account it may be more convenient to perform the experiment with *Phaseolus*, and allow the plants to grow ten days before taking the weights.

325. Anaërobes. It has been shown in a previous experiment

that certain plants may exist with only intramolecular respiration during the seedling stage, and many Phanerogams are probably anaërobic to a similar extent. An increasing capacity for non-atmospheric respiration is to be found among the lower forms, especially those devoid of chlorophyl. Among these forms all gradations may be found between aërobes and anaërobes, and some of them may spend extended periods with, and without oxygen. Some species have been demonstrated to be able to exist many generations without access to free oxygen, but actual proof that any organism is capable of indefinite life without this supply has not been adduced, although it seems quite probable from all the evidence at hand. Many of the anaërobic organisms are capable not only of carrying on intramolecular respiration but also of producing fermentation in a medium in which they live. Fermentation may be due to the direct action of the protoplasm, or to that of substances, enzymes, secreted by it. It may occur in the tissues of the plant, or the enzyme may be excreted, and set up disintegration in the medium in which the plant lies. The latter is the case in nearly all of the simpler forms like bacteria and fungi. Such fermentations may be accompanied with the evolution of carbon dioxide and other gases, or not, and are generally characterized by an enormous liberation of energy, and some heat. As pointed out in a previous paragraph, the fermentation may be simply for the purpose of rendering substances assimilable and thus constitute digestion.

326. Estimation of the Amount of Carbon Dioxide Given off and Oxygen Absorbed During Respiration. Germinate about 200 cc. of seeds of wheat, or corn, and when the roots are about 3 cm. in length place in a suitable vessel and set in the receiver used for the volumetric determination of the interchange of gases in photosynthesis (Fig. 118). Insert a naked bulb thermometer in the seeds, make the proper connections and determine the proportion of carbon dioxide and oxygen present at the beginning of the test. Allow the preparation to stand for four hours and make a second estimation. If the capacity of the bell-jar cover-

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ing the seeds has been calculated the exact amount of the gases interchanged may be found.   The Bonnier and Mangin apparatus may be used instead of the one referred to above.

327. Estimation of Atmospheric Gases with Bonnier and Mangin Apparatus.   Fill a test-tube with mercury and support mouth downward in a small receiver full of mercury (Fig. 127, *l*).   Connect a short bent tube with the outlet of the receiver containing the germinating seeds.   Run water into the receiver displacing the air, and as soon as that in the outlet tubes has been thrown out run the tip under the rim of the inverted test-tube and allow a few cc. to escape into it.   Close the receiver and transfer the test-tube to
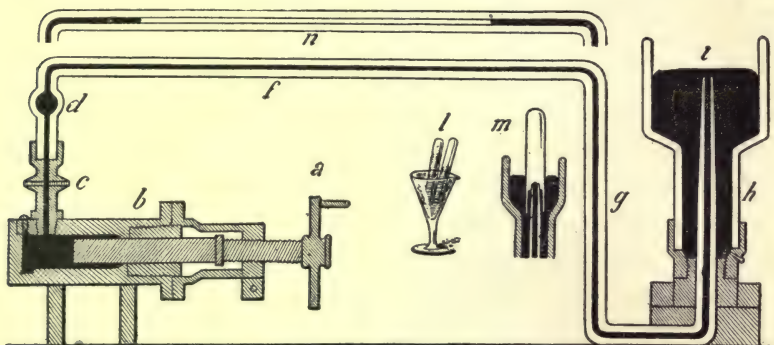
FIG. 127. Apparatus for determination of atmospheric gases designed by Bonnier and Mangin.   *a*, wheel with handle attached to a piston with threads on its surface : the inner end of the piston projects into the cavity of a cylinder *b*, which is filled with mercury ; revolutions of the piston cause movements out of the cylinder into the graduated glass tube *fg*, and vice versa.   *c*, metallic fitting, connecting graduated tube with cylinder.   *d*, bulb in glass tube.   *f*, portion of glass tube, calibrated.   *g*, U-shaped portion of glass tube the outer end of which lies underneath the surface of the mercury in the vessel *h*.   *m*, showing method of placing test-tube containing gas to allow the gas to be drawn into the graduated tube.   *n*, showing the portion of the graduated tube which should be filled by the gas in a test.   *l*, small receiver suitable for containing the solution used in absorbing gases.   A similar form is suitable for mercury over which the test-tubes may be filled with gas from the bell-jar containing the plant.   After Belzung

the bowl of the measuring apparatus, holding the thumb over the mouth of the inverted tube enclosing a layer of mercury and the gas to be tested.   Press the test-tube downward over the capillary

tube until its tip projects into the gas (*m*). Turn the crank wheel contrary to the movements of the hands of a watch, until the gas fills about half of the free vertical portion of the capillary tube. Remove the test-tube and force the gas on into the horizontal portion of the tube (*f*). Read carefully the amount of the gas in the tube (*n*). In the same experiment all readings should be taken in the same part of the scale, as the tube may not be exactly calibrated. Force the gas back into the bulb *d*. Prepare a 25 per-cent. solution of potassium hydrate in distilled water, in a test-tube over mercury. Introduce enough of this solution into the capillary tube to about fill half the vertical portion. Remove the test-tube and force both the gas and solution into the bulb of the vertical portion at the left. Let remain until the potassium solution has absorbed all the carbonic acid gas : a few minutes will suffice. Bring the gas back in the tube and take another reading ; the difference between the readings will be the amount of carbon dioxide present.

The amount of oxygen can now be determined by introducing a mixture of 1 part of 25 per-cent. solution of potassium hydrate and 6 parts of 60 per-cent. solution of pyrogallic acid and after manipulating as before, taking another reading. In atmospheric gases the remainder may be taken to be nitrogen. The absorption solutions should always be freshly prepared.[1]

328. Influence of Temperature Upon Respiration. Prepare a second lot of seeds as in 298 and when ready to place in the receiver, wash with cold water for five minutes and then set in place. Fill the dish which is used to receive the displacing fluid of the burette with a mixture of pounded ice and salt. Insert thermometer in seeds, and close all fittings. Take readings of the temperature every half hour for four hours. Make an estimation of the carbon dioxide and oxygen present at the beginning and close of this time. Compare with results obtained in 298. Marked results may be obtained in three or even two hours.

[1] Bonnier and Mangin. Recherches sur la respiration et la transpiration des champignons. Ann. Sc. Nat. Bot. **6**, **17** : 210. 1884. Also, Recherches sur la respiration des tissues sans chlorophylle. Same journal, **6**. **18** : 293. 1884.

Take the receiver away, warm the seeds by immersion in water at 35 to 38° C. for five minutes, then replace and set large flasks or dishes full of hot sand inside the receiver. Make connections and estimate the proportion of oxygen and carbon dioxide present at the beginning and three hours later. Make temperature records. Compare results at the three temperatures.

329. Estimation of the Respiratory Quotient. The following method designed by Puriewitsch will be found most convenient
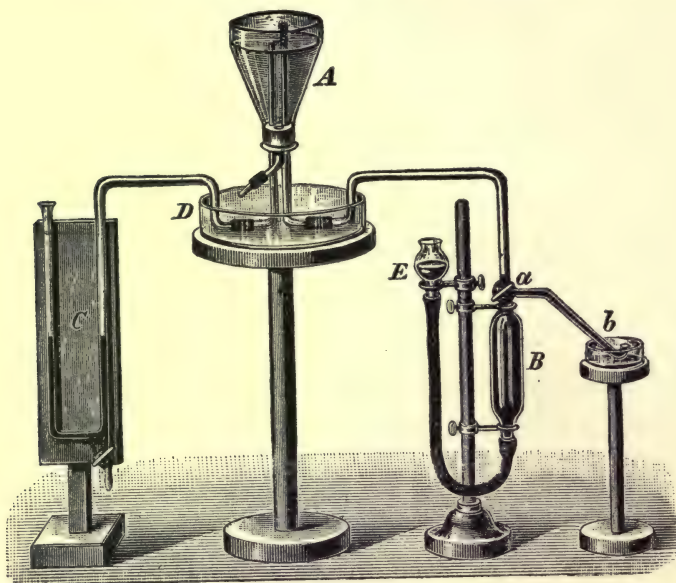
FIG. 128. Apparatus for the determination of respiratory quotient under the influence of various nutrient solutions. *A*, receiver, an Erlenmeyer flask, into which projects three glass tubes. The shortest tube merely passes through the stopper and is closed externally by a section of rubber tubing and a pinchcock. A longer tube extending just above the surface of the fluid in the flask is connected at *D* with a manometer *C*. *B*, burette, with a three-way stopcock at *a*. The lower end of the burette is connected by a strong rubber tube with a short thistle tube, *E*. The free arm of the stopcock *a* is bent and the end curved upward, the tip being immersed in a small dish of mercury at *b*. After Puriewitsch.

for estimation of the respiratory quotient. Secure an Erlenmeyer flask of a capacity of about 200 cc. and provide it with a

rubber stopper with three holes.   Insert through one of the aper-
tures a suitable glass tube which reaches almost to the bottom of
the flask, and has the outer short end bent at right angles.   A
second tube of the same pattern but which does not extend so
near the bottom as the first by a cm. is put in place in a second
hole, and a short tube bent at 45° is put in the third.   Invert
the flask and attach a funnel to the shortest tube and pour into
the flask 100 cc. of a nutritive solution which has been thoroughly
infected with spores of *Aspergillus*.   The nutritive solution should
fill the inverted flask to within 2 cm. of the bottom, and should
not reach the ends of the long tubes.   Allow the preparation to
stand for a day or two until a mycelial layer has been formed on
the solution, then open the outlet and allow the solution to flow
out slowly, leaving the mycelium attached to the walls of the
flask.   Introduce distilled water and let it stand for a few min-
utes to take out the traces of remaining nutritive fluid.   Remove
the water and introduce the substances the influence of which is
to be tested.   First put in a 2 per-cent. solution of dextrose, fill-
ing the flask to the original level and, after the preparation has
stood an hour or two, draw fresh air through the longer tubes by
means of a filter pump, and then fit the flask with a manometer
attached to one of the tubes and an apparatus for withdrawing
air for analysis to the second (Fig. 128).   The latter consists of a
three-way stopcock with two long arms, attached to a burette
containing mercury.   Fit the short arm of the three-way tube to
the upper end of the burette *B*, and bend one arm so that it may
be immersed in mercury in the small dish *b*.   Attach the burette
to a suitable support and raise the bowl at the end of the flexible
arm until the mercury rises above the stopcock at *a*.   Now at-
tach the other arm of the tube to the free end of the tube pro-
jecting from the flask.   To do this it should be suitably bent
three times at right angles.   Fill the dish *D* with water and at-
tach the mercury manometer *C*, making all joints secure by wire
bindings.   This may be tested by lowering the bowl *E* with the
stopcock open, drawing the mercury up in the manometer arm.

Close the stopcock a and if the level of the mercury in the manometer arm remains stationary the joints are safe.   Now restore the mercury above the stopcock a and allow the preparation to stand for two or three hours.   Open the stopcock at a, leading into B, and draw all of the air out of the tubes, then turn the cock and force it into the open.   Air for testing now may be withdrawn in the same manner and forced into a test-tube filled with mercury ; analyze with the Bonnier and Mangin apparatus (Fig. 127).   1 to 2 cm. of the gas will be sufficient and several calibrations should be made from it.   The tests will give the data for the respiratory quotient.   Disconnect and draw air through the preparation for a few minutes, then allow to rest two or three hours, and make a second or third test.   Comparative tests should be made at identical temperatures.

An error in such tests consists in the absorption of the watery vapor in the air by the potassium solution, but this will not greatly vitiate comparative tests.

After one calibration has been made, disconnect the apparatus and wash out the dextrose from the culture and replace with 5 per-cent. solution of tartaric acid, and repeat tests.[1]

330. Respiration of Oily Seeds.   Soak about 200 cc. of seeds of hemp for a few hours in water, then place in the receiver, make an estimation of the proportions of carbon dioxide and oxygen at the beginning of the experiment and four hours later.

331. Respiration of Peas.   Place about 200 cc. of germinated peas in the receiver as above and estimate the oxygen and carbon dioxide at the beginning and end of a four-hour period. Interesting results may also be reached by using a number of flowers, mushrooms, or etiolated plants instead of seeds.

332. Production of Heat in Respiration.   Secure two strong cardboard boxes of a capacity of about 600 cc. and cut slits in the sides so that the bulbs of a differential thermometer may be

[1] Puriewitsch, K.   Physiologische Untersuchungen über Pflanzenathmung.   Jahrb. Wiss. Bot. **35** : 573.   1900.

Palladine, M. W.   Influence de la nutrition par diverse substances organiques sur la respiration des plantes.   Rev. Gen. Bot. **30** : 18, 93.   1901.

enclosed in the center of each. Support the boxes properly.
Germinate enough seeds of *Pisum* to fill both boxes. When the
roots are a centimeter long, fill one of the boxes with seedlings
which have been killed by boil-
ing water and then placed in a
separate germinator to cool.
Fill the second box with normal
seedlings. Both lots must offer
the same degree of moisture.
Place the apparatus where it may
not receive sunlight and both
lots of seeds will receive the
same temperature from the out-
side. Lay a piece of moistened
filter paper over the top of the
seedlings. Note the height of
the columns of colored spirits
in the arms of the tube at inter-
vals of an hour (Fig. 129).
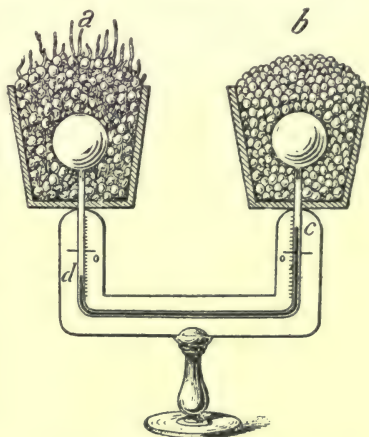
FIG. 129. Measurement of heat pro-
duced in respiration, by differential ther-
mometer. *a*, cardboard box containing
growing seedlings. *b*, box containing
germinated seedlings killed by immer-
sion in hot water and then cooled. The
expansion of the vapor in the bulb in the
living seedlings has driven the column
of spirits down in the arm below and up
into the other arm of the thermometer,
which has not been similarly affected.
After Belzung.

**333. Production of Heat in
Fermentation.** Pour 250 cc. of
Pasteur's solution into an Erlen-
meyer flask, and a similar
amount of distilled water in a
second. Place 5 g. compressed
yeast in each flask, and close the
mouth of each with cotton wool through which passes a delicate
thermometer so that the bulb is immersed in the culture solution.
Place both flasks in a steady temperature of about 22 to 25°
C. Compare the temperature of the two flasks after fermenta-
tion has set up. This experiment may also be performed with
the differential thermometer. Secure two rubber stops of a
diameter greater than the bulbs. Make perforations the size of
the thermometer tubes, and a cut from one side into the per-
forations. Place around the tubes, and fit to them large tubes,

such as lamp chimneys in such manner that the rubbers form the bottoms of the cylinders. Fill one with water and the other with solution for fermentation. Note results hourly.

334. Products of the Fermentation of Sugar. Make 1,000 g. of Pasteur's solution by adding to 838 cc. of water, 150 cc. of grape-sugar, 10 cc. ammonium tartrate, 2 g. each of magnesium sulphate, calcium phosphate, and 2 g. of potassium phosphate. Pour 250 cc. of the solution in a glass cylinder and add 5 g. compressed yeast and keep at a temperature of about 25° C. Cover the vessel with a glass plate, or fit with a cork stopper. Test the air above the liquid for carbon dioxide a few hours later. Place some of the solution in a distilling apparatus and test the condensation for alcohol. Make a strong solution of potassium bichromate in water, and add a few drops of sulphuric acid. Pour a few drops of this mixture into a test-tube containing some of the distillate. The presence of alcohol will be denoted by the appearance of a greenish color. The fermentation tested above is produced by an enzyme excreted by the yeast cells. Tests of the effects of anaesthetics may be made.[1]

335. Digestion. Digestion in plants includes the processes by which certain insoluble and non-dialyzable substances are converted into diffusible form, and other material is changed in such manner that it may be absorbed and used by protoplasm. These changes embrace hydrolyzation, and oxidation and splitting up of the various food substances. Digestion of some substances may be carried on by the direct action of protoplasm, or may be effected by the catalytic action of certain proteid secretions termed enzymes. All enzymatic effects are not productive of plastic or diffusive material, and hence are not to be included in digestion. A number of these enzymes have been isolated and

[1] Bokorny, Th. Empfindlichkeit einiger Hefenzyme gegen Protoplasmagifte. Wettendorfers Zeitschrift f. Spiritus-Industrie. September, 1900.

For a comprehensive account of fermentations by minute organisms see Duclaux, E. Traite de Microbiologie, 2 : 1899, and 3 : 1900.

Jörgensen, A. Micro-organisms and fermentation. Trans. Miller & Leunholm. 3d Ed. 1900.

it is found that each one of them is capable of acting on but one or a few substances. The characteristic action of an enzyme is its power to induce chemical changes in an amount of material vastly disproportionate to its own bulk. Diastase is able to hydrolyze ten thousand times its own bulk of starch and invertin may convert a hundred thousand times its bulk of cane-sugar into invert sugar. Enzymes are quickly destroyed by the blue violet rays of light and are not active below a temperature of freezing, and each reaches a specific optimum at a point between 30 and 50° C. and all are destroyed at a temperature below 100° C. when moist.

336. Classification of Enzymes. The following classification of enzymes is made by Green on the basis of the character of the material acted upon.[1]

ENZYMES acting upon carbohydrates producing soluble sugars. Diastases attack starch and related substances. Inulase decomposes inulin.

CYTASE, hydrolyzes the celluloses of which walls are composed.

ENZYMES which transform sugar of the biose type into simpler sugars, usually hexoses. Invertase attacks cane-sugar. Glucose splits up maltose.

ENZYMES which decompose glucosides, of which emulsin and tyrosin are examples.

ENZYMES which decompose proteids, among which are pepsin and trypsin probably identical with the substance of the same name in animal digestion.

ENZYMES which produce jelly-like substances from soluble liquids, including rennet, thrombase, and pectase.

ENZYMES which attack oils and fats of which but one has been determined, lipase.

OXIDASES which oxidize various substances inclusive of coloring matters. The best known oxidases are laccase and tyrosinase.

Many other fermentative processes that can not be ascribed to

[1] Green, J. R. Fermentation. Cambridge. 1899.

the direct action of living matter, or to any of the above enzymes have been observed, and it is probable that the above list will be greatly extended by further investigations.

337. Origin and Distribution of Enzymes. Some enzymes may be formed in almost any cell of a plant, in which their origin may not be traced to any special plastid. In other instances specialized cells are differentiated for the chief purpose of secreting these substances. Such glandular cells may be seen as forming the aleurone layer in the seeds of grasses and other monocotyledons, and also the epithelial layer of the cotyledon or scutellum. The secretion of an enzyme seems to be preceded by the formation of a granular substance known as zymogen, as a result of the joint action of the nucleus and cytoplasm in such glandular organs, although it is not possible to observe all stages of the process in every instance. Extracted enzymes are dialyzable with difficulty, and may not pass out of the glandular cells in which they are formed by diosmose. Their discharge must be effected by a passage along interprotoplastic threads, or they may pass through the walls and plastic membranes by filtration pressure in an emulsified condition in the same manner that oils and waxy substances accomplish translocation. The last named method is the only one by which the enzymes of bacteria and other unicellular organisms could be excreted. In seeds the enzyme must pass many membranes to reach all parts of the storage tissue containing food which must be digested. It is possible that zymogen might pass a wall or membrane and then become converted into an enzyme.[1]

338. Localization of Digestion. Digestion occurs in all protoplasts in which reserve food accumulates. The products of synthetic processes may be of such nature as to be capable of assimilation without change, which would be a desirable and economical arrangement. The preponderating constructive capacity of the plant however, furnishes it with a surplus which is changed to

[1] Laurent, J. Sur l'exosmose de diastase par les plantules. Compt. Rend. **131** : 848. 1900.

condensed and insoluble forms for storage. All such substances must undergo digestion before leaving the cell in which they are deposited. When a stream of material sets in from a place of storage, or formation, to a tissue using or restoring the translocated food, it often occurs that the material is not used so rapidly as it is moved, in which case it is converted into insoluble forms and stored in transit in convenient cells. Before such substances stored by the wayside can be moved farther they must undergo digestion again. An instance of this is afforded by starch formed in mesophyl cells in leaves. It undergoes digestion into maltose before leaving the cell, and may be converted back into starch in the next, and so on numberless times before finally being assimilated, and yielding its energy to living matter.

Simple organisms like bacteria and fungi carry on extra-cellular digestion by excreting enzymes which act upon the substances in the medium in which they live, and the results of the digestion are absorbed. Parasitic forms use an enzyme to dissolve the walls of the host and allow them to penetrate the protoplasts, and the extending tubes of pollen cells are provided with a similar means of boring down through the style of the flower.

Extra-cellular digestion is also effected by species of the Nepenthes family in which a proteolytic enzyme is excreted by the glandular cells lining the pitchers, and the fluid contained in the pitchers is thus enabled to digest the bodies of animals entrapped. *Dionaea*, *Drosera*, and other carnivorous species show a similar adaptation.

The embryos of a large number of species are enclosed with an endosperm containing stored food, which is digested partly by the excretions of glandular cells of the endosperm, and of the embryo. The translocation of stored food from seeds, bulbs tubers, etc., affords the most interesting examples of digestion in which but little investigation has been carried out.

339. Digestion of Starch. Two kinds of diastase have been isolated that act hydrolytically upon starch. One is known as the diastase of translocation, and is found in germinating seeds

and all parts of the shoot as well as in the lower forms of plants. The other, diastase of secretion, is to be found chiefly in germinating seeds and does not appear until after germinating is well under way.

Many theories have been advanced as to the chemical action ensuing during hydrolysis of starch, the best supported of which seems to be that of Brown and Morris [1] that starch is composed of a number of dextrin groups, which are successively split off and converted into maltose.   During the process many substances appear in the reactions.

Diastase of secretion is most active at 50–55° C., and its action on starch grains results in their irregular corrosion, giving them a jagged outline during dissolution.   It is formed most abundantly in the epithelial layers of certain embryos, although some of it is present in the aleurone layer, which contains mostly diastase of translocation, and both are accompanied by cytase. Diastase of translocation is most active at temperatures of 40–

FIG. 130. Section of portion of scutellum of barley showing the secreting epithelium.   After Greene.

50° C., and it attacks the layers of the starch granule uniformly so that its outward form is preserved until almost completely dissolved.

340. Enzymatic Glands of Seeds. Secure some sound seeds of barley, oats, rye, wheat, corn or *Arisaema* or any arum and soak for a few hours in water. Cut thin cross sections through the seed in such manner that the scutellum, or the upper part of the cotyledon will be sectioned at right angles.   Treat some of the preparations with nuclear and others with cytoplasmic stains. Note the character of the layer immediately underneath the testa, and also the outer layer of the embryonic organ in contact with

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[1] Green, J. R.   Fermentation.   P. 32.   1899.

[2] Krabbe.   Untersuchungen ueber das Diastaseferment unter specieller Beruck-sichtigung seiner Wirkung auf Stärkekörner innerhalb der Pflanze.   Jahrb. Wiss. Bot. **20** : 520.   1890.

the endosperm. Test both layers for proteids (see Zimmerman's Botanical Microtechnique). Germinate some of the seeds and after the roots are a centimeter in length cut sections as before. The germination should be carried on in a thermostat at a temperature of at least 22° C. or perhaps 30° C. Compare the appearance and structure of the secreting layers with that of the resting seed, and look for changes in the contents of the cells near them.

341. Action of Secretion from Scutellum on Starch. Grate the white portion of a potato finely, and fill the cavity in a dozen culture slides. Carefully dissect out the embryos of an equal number of seeds of corn that have laid between folds of damp cloth, or in a germinator, for two days. Lay one of these embryos with the scutellum downward in each of the masses of grated potato in the slides, and put all of the preparations in a moist chamber at a temperature of 40° C. Fill one of the slides with grated potato only and also lay in moist chamber. Examine the grated potato with the microscope and note the appearance of the starch grains. Test with iodine, and note exact color reaction. Test the grated material for sugar with Fehling's solution (220). Now institute a series of tests at intervals of an hour, to ascertain the beginning, and course of the hydrolysis of the starch. This will be indicated by a change in the color reaction with iodine, due to the intermediate products, and no color reaction will be given after all of the starch has been converted into maltose. Test final solution for sugar with Fehling's solution. Cut sections of a seed attached to young plantlets of corn and note the different staining reactions with iodine.

342. Digestion of Cellulose. Cellulose walls of cells are in reality made up of quite a number of substances including some pectoses. The digestion of the celluloses appears to be accomplished by cytase and other enzymes. The process is one of hydrolyzation and some form of sugar is the principal product. Cytase is probably most active at a temperature of 35–40° C., and is destroyed at 70° C. It is formed by seeds, and is espe-

cially evident in the aleurone layer of seeds of some grasses, although found in epithelial cells of embryos of palms, in which reserve material is deposited in the seed in the form of thickenings of the cellulose walls.   It passes from the embryo to the endosperm by dialysis.

Cytase is formed by many parasitic plants which use this means of dissolving the cellulose walls of the host plants and thus gain access to the protoplasts.   Lignified and suberized membranes are not attacked, and changes in the membrane may be an adaptive device by which a possible host avoids penetration by fungi and bacteria.[1]

343. Action of Cellulose Dissolving Enzymes.   Germinate a number of seeds taken from the ordinary dried dates of commerce, which will need about six weeks.   Two weeks after germination has begun, cut cross sections of the seed and embryo.   Note the structure of the epithelial cells of the absorbing organ formed from the cotyledon.   Stain with iodine.   Note the condition of the walls nearest the absorbing organ.   Stain a fresh section with chlor-zinc iodide and note the color reaction in the contiguous and distal regions.   Boil a fresh section in Fehling's solution and note the presence of a reducing substance, probably sugar in the region nearest the embryo.

Follow the action of cytase on the membranes nearest the aleurone layer, and the epithelial layer of the cotyledon in the plants used in the last experiment.

344. Digestion of Sugars.   A large number of kinds of sugar occur in the plant, and at the present time six different enzymes have been discovered, each of which acts upon but one or few forms of the carbohydrate.   Invertase, which has the power of inverting or converting cane-sugar into glucose and fructose is the most important.   It is found in all parts of the higher plants including pollen grains, and is also formed by yeast, moulds and

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[1] Newcombe, F. C.   Cellulose enzymes.   Annals of Botany. **13** : 49.   1898.

Kohnstamm, P.   Amylolytische, glycosidespaltende, proteolytische, und cellulose-losende Fermente in holzbewohnende Pilzen.   Beih. Bot. Centralb. **10** : 90.   1901.

other fungi. It appears to be excreted for the purpose of extra-cellular digestion in the lower forms. The purpose of inversion of cane-sugar is not known.[1]

Maltose and other sugars produced in the digestion of various carbohydrates may be attacked by other enzymes beside those named, at the time of their production, and as several fermentations may proceed simultaneously the chemical results are somewhat complicated.

345. Digestion of Proteids. A number of enzymes are known which act upon vegetable proteids, chiefly of the type of trypsin. This exerts a hydrolytic action on proteids breaking them into substances not to be classed as proteids, in contradistinction to pepsin, a proteolytic enzyme abundant in animals and perhaps present in a few plants. The products of pepsin fermentation are soluble proteids, but the constitution of the proteid molecule is so little known, that no statement can be made as to the chemical changes ensuing during either tryptic or peptic fermentation.

Tryptic fermentation usually ensues in the cells in which the enzyme is secreted, and it has been found in many seeds and fruits, accompanied in some instances by pepsin. It is excreted and accomplishes digestion outside the body in bacteria, fung and the carnivorous plants however.

346. Digestion of Albumen by Drosera. The following experiment by F. Darwin demonstrates the fermentative action of the enyme secreted by the glandular hairs of *Drosera*.[2]

Cultivate a number of plants of any convenient species of *Drosera* in shallow dishes or pots filled with sphagnum in a temperate greenhouse. Cut a number of cubes of the white of a hardboiled egg about a millimeter in diameter, and select a few with sharp corners and edges. Place one or two of these cubes of albumen on each of several fresh young leaves where they may be enclosed by the tentacles and lay a few of the cubes on the moss near the plants. Examine the cubes a day later with a

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[1] See Green, J. R. · Sugar splitting enzymes. Fermentation. p. 105. 1899.
[2] Darwin and Acton. Physiology of Plants. p. 64. 1894.

hand lens, and again on the second day. The beginning of digestion of the albumen will be denoted by the loss of sharpness of the edges of the egg material and that after a time the outer portions are converted into a transparent fluid.[1]

347. Digestive Action of Nepenthes. Suck up a few cm. of the clear liquid in the pitchers of any species of *Nepenthes* and place in a watch glass. Immerse one or two cubes of white of an egg in the albumen, cover and set in a thermostat at 35° C. Prepare a second, adding a small drop of dilute hydrochloric acid to the liquid. Note the appearance of the cubes a day later. The digestive enzyme is variously held to be a trypsin or pepsin.[2]

348. Glands of the Pitchers of Nepenthes. Cut a cross section of the glandular region of the pitchers and examine the structure and character of the contents of the glands. It is to be noted that the pitchers of *Sarracenia* have not yielded any enzyme in investigations made upon them, and they are supposed to absorb the products of bacterial fermentation, and simple decomposition of the material finding its way into the pitchers.[3]

349. The Clotting Enzymes. The clotting enzymes represented in plants by rennet and pectase differ from those previously discussed in not being concerned with digestion, or indeed to any great extent with processes in the chain of general metabolism. Their characteristic action consists in the coagulation of substances from the solutions in which they are found, forming gelatinous masses which later undergo contraction and hardening, becoming semi-fibrous. The formation of the jelly-like material may be accompanied by the separation of substances as soluble as the original solution. Calcium has been found as an

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[1] Huie, L.   Action of the glands of *Drosera*.   Quart. Jour. Roy. Mic. Soc., Vol. **39**.

[2] Clautriau, G.   La digestion dans les urnes de Nepenthes.   Mem. Cour. e. a. Mem. pub. p. l'Acad. Roy. d. Belg. **59** :' 1900.

Vines. The proteolytic enzyme of Nepenthes. Annals of Botany. **12**: 545. 1898.

[3] MacFarlane, J. M.   Observations on pitchered insectivorous plants.   Annals of Botany. **7** : 403. 1893.

Butkewitsch, W.   Ueber das Vorkommen eines proteolytischen Enzyms in gekeimten Samen und seine Wirkung.   Zeitschr. f. Physiol. Chemie. **32** : 1. 1901.

invariable accompaniment of the activity of the clotting enzymes, although no actual connection of its presence with the chemical changes has been demonstrated.

Rennet is found in the seeds and fruits of a number of plants, and is also present in some bacteria. It is capable of acting in acid, neutral or alkaline solutions.

350. Pectase. Pectase is almost universally distributed in plants and is most abundant in the growing regions. Certain changes which take place in the cell-membranes during the life of the protoplast are supposed to be due to its action. The cellulose of the wall is accompanied by the presence of other substances among which are pectine and pectic acid, which may be derived from pectose. Pectine is most abundant in the membranes of younger cells, and compounds of pectic acid increase in abundance with age ; this action however, ensues only before the beginning of suberization and lignification.

The middle lamella of cell-membranes has long been known to differ chemically from the layers contiguous to the ectoplasm, and the hypothesis has been advanced that the action of pectase converts pectine into pectic acid, which slowly passes outwardly through the wall from each protoplast by exudation pressure, and combines with calcium salts to form calcic pectate, which is deposited on the external surface of the wall. At points in which the walls of contiguous cells are in contact a layer of calcic pectate formed from contributions from both cells would be laid down as the middle lamella. A free layer of the substance would result where the cell abuts on an intercellular space. This theory is supported substantially by the fact that the middle lamella may be dissolved by the reagents in which calcic pectate is known to be soluble.

351. Oxidases. The oxidases include a number of enzymes which have the power of producing oxidation in various compounds including fatty acids and sugars. Although no actual demonstration has been made, it is supposed in some cases that the energy liberated in the process becomes available to the pro-

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toplasm and the process constitutes one form of respiration. Certain of these enzymes might also operate to break up hydrogen peroxide when formed in the plant and thus prevent its poisonous action on the protoplasm.[1]   Two groups may be distinguished, viz., the oxidases proper, which are destroyed in aqueous solutions by temperatures of 65° to 70° C., and the peroxidases which disintegrate at 80° to 85° C.   Both act most readily in slightly acid solutions : Catalase, however, does not fall in either of the above groups from which it varies in many properties.

Oxidizing enzymes are widely distributed in plants, and do not decompose readily upon the death of the cells, but produce many post-mortem changes in the constituents of the plant, and may even pass out into the soil upon the complete decay of the plant remaining unimpaired for long periods.   Various bleaching and curing processes, development of flavors, preparation of indigo, fermentation of tobacco, and silage, and other commercial operations are dependent upon the action of these substances.   The well-known coloration, or browning of the exposed surfaces of apples and other fruits is an example of the action of oxidases.

The development of an excessive amount of oxidase in plants subject to defective nutrition, and many kinds of cultural treat-

[1] Loew, O.   Curing and fermentation of cigar leaf tobacco.   U. S. Dept. of Agriculture.   Report No. 59.   1899.

Loew, O.   Physiological studies of Connecticut leaf tobacco.   U. S. Dept. of Agriculture.   Report No. 65.   1900.

Woods, A. F.   Brunissure of the vine and other plants.   Science, N. S. 9 : 508. 1899.

Woods, A. F.   The destruction of chlorophyll by oxidizing enzymes.   Centralbl. f. Bakteriol. Parasitenk. u. Infektionskrankh. 5 : 745.   1899.

Woods, A. F.   Inhibiting action of oxidase upon diastase.   Science, N. S. 11 : 17.   1900.

Loew, O.   Catalase : a new enzyme of general occurence.   U. S. Dept. of Agriculture.   Report No. 68.   1901.

Woods, A. F.   Mosaic disease of tobacco.   Abstract, Science, N. S. 13 : 247. 1901.

Green, J. R.   The soluble ferments and fermentation.   Chapter 19.   1899.

Aso, K.   A physiological function of oxydase in kaki-fruit.   Tokyo Bot. Magazine, 14 : 179.   1900.

ment results in an oxidation of chlorophyl, producing yellow foliage.   The pale regions in variegated leaves are also associated with the action of these enzymes on the growth of the cells.   To this action also some of the autumnal coloration of leaves may be ascribed.   The " mosaic disease " of tobacco, and the " brunissure of vines " are examples of the development of unusual amounts of oxidases in the plant.   Some of the pathological phenomena in these and other diseases of the plant are due to the interference of the oxidases with the digestion of starch, since it is found that oxidases inhibit the action of diastases, and thus prevent the translocation of this substance (339).   To this fact may be ascribed he accumulation of starch in injured portions of leaves due to animals and fungi.

352. Demonstration of the Presence of Catalase and Other Oxidizing Enzymes.   Catalase may be found in almost all animal and vegetable tissues.   The material to be tested should be finely divided and put into a test-tube with enough water to cover it, and a few drops of hydrogen peroxide added.   If catalase is present bubbles of oxygen will be quickly formed, and will continue to be given off until all the oxygen in the solution is liberated.

Some of the ordinary oxidases and peroxidases may be demonstrated by moistening the freshly cut surface of the tissue to be tested with a two per-cent. solution of gum guiac in 95 per-cent. alcohol.   If the more active oxidizing enzymes are present (oxidases) the cut surfaces will turn blue.   The addition of a little peroxide of hydrogen will increase the intensity of the color if weaker enzymes of this class (peroxidases) are present.   Enzymes of the three groups, viz., catalase, oxidases and peroxidases usually occur together in most plants.[1]

[1] Vines, S. H.   On Leptomin.   Annals of Botany. **25** : 181.   1901.

XIII. GROWTH

353. Volume Relations of Protoplasm. Increase in size, and physiological differentiation of a plant depend upon the increase in size, and capacity for morphological differentiation of the cells of which it is composed. The ultimate volume of a protoplast is limited by the physical characteristics of living matter, and the degree of differentiation it may show in its various organs is influenced by forces resident in the chemical relations of these units. A unicellular organism is therefore incapable of attaining a bulk beyond that possible to a body with the viscosity of its protoplasm, or a degree of complexity beyond that offered by the diverse nature of its nucleus, cytoplasm and plastids. An organism may increase its capabilities in both features if it is composed of a number of fused protoplasts to form a coenocyte with several nuclei, numerous plastids and a large mass of cytoplasmic material. The bulk and functional development of higher organisms must rest, however, upon the multiplication of the cells, and their enlargement to the physiological and physical optimum of size and efficiency.

354. Purpose of Multiplication of Cells. The cell, in simpler organisms, increases in volume until the approximate limit is reached, when it divides by various methods into two or more cells, which in turn repeat the process. This action gives rise to linear series of cells of the same degree of differentiation.

The spore or egg cell of the higher plant undergoes division and re-division in such manner as to lead to the formation of a number of protoplasts, some of which lose the power of further division or multiplication, and assume certain functions for the performance of which they show more or less specialized morphological characters. A fraction of the products of division retain

the original capacity of division and thus constitute *generative layers, cambium,* or *phellogen,* or growing regions.

The increase in amount of living material of the cells formed by the generative layers, and the consequent increase in volume of these cells, accompanied by more or less morphological differ-
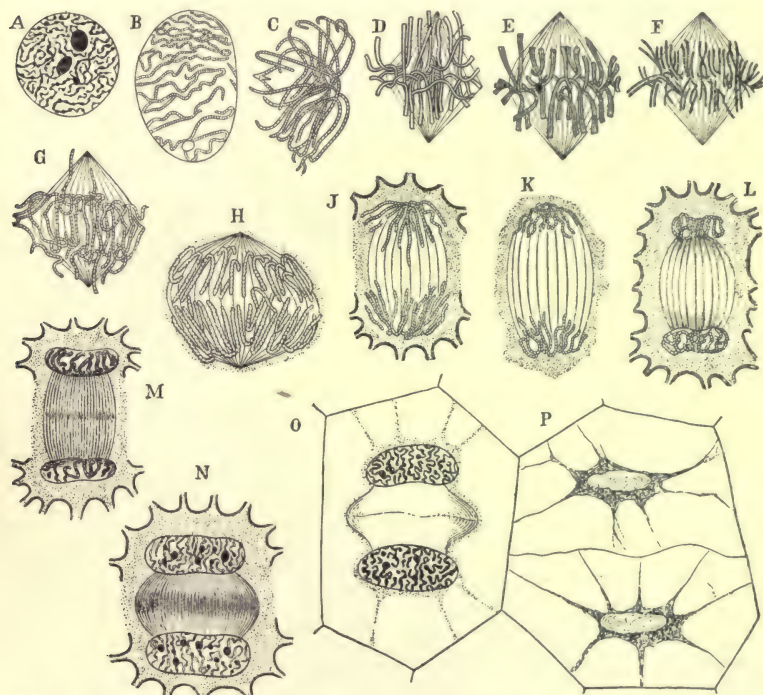
FIG. 131. Changes in nucleus during mitotic division. *A,* earliest observable stage, showing a coarsely granular thread of filament. *B,* a later stage in which the thread is arranged in parallel segments (chromosomes), and the cytoplasm (not shown) begins to arrange itself around two poles. *C, D,* arrangement of the segments or chromosomes in the nuclear plate, and showing the spindle fibers. *D, E, F,* showing longitudinal division of the chromosomes. *G, H,* separation of the newly formed chromosomes, and movement toward the poles of the nuclear spindle. *J–P,* stages in which the daughter chromosomes collect and fuse into the nuclear substance of the daughter nuclei. *M, N,* the spindle fibers connecting the daughter nuclei remain, and midway between the nuclei may be seen an aggregation of finely granular material, which finally fuses together in a continuous membrane, the cell-plate, in *O,* and forms a wall in *P.* After Strasburger.

entiation in the transformation of such cells into permanent tissues, constitute the essential features of growth.

355. Cell Division. Division of a protoplast in the multiplication of cells is accomplished by a separation of the nucleus, cytoplasm and plastids into physiologically equivalent parts, which are organized to carry out the functions of the original cell. Two general methods, with regard to the action of the nucleus during the process, may be distinguished, viz., mitosis and amitosis.

Division of the cell with mitosis is characterized by a chemical and physical transformation of the nucleus, in which the chromatin assumes the form of rods and increases in staining power. The limiting membrane of the nucleus disappears, and much of the cytoplasm is involved in the evolutions of the nucleus. The chromosomes split longitudinally, and the halves separate and collect in equal portions at poles of the spindle, being connected by a number of interzonal fibers. This separation of the components of the nucleus is generally followed by the division of the cell by the formation of a plate or wall midway between the groups of chromosomes and finally extending to the periphery of the cell. Meanwhile the groups of chromosomes are organized as daughter nuclei and quickly assume the structure of the original nucleus (Fig. 131).[1]

Division of the cell with direct separation of the nucleus into two parts in amitosis has not been so thoroughly investigated. It appears, however, that the nucleus is divided by a simple constriction cutting through the nucleus, which undergoes no structural changes of the chromatin, or reticulum, preliminary to this process. Various intermediate stages between mitotic and amitotic division of the nucleus have been observed (Fig. 132).

It is held by many writers that mitotic division is characteristic of vigorous actively growing cells, and that the equal division of the chromosomes between the daughter nuclei is necessary to insure the proper transmission of the parental qualities to the two daughter cells, and that direct division is to be found

[1] See Wilson, E. B. The cell in development and inheritance. 65. 1900.

only in degenerating tissues decreasing in energy and approach-
ing the end of the senescent period.   Recent investigations have
shown however, that the method of division is highly susceptible
to external influences and that mitosis may be inhibited for gen-
erations of cells, and that the descendants by amitotic division
exhibit no differences from those resulting from division with mi-
tosis of the nucleus.[2]

356. Growth and Senescence of the Cell.   After a daughter
cell has been formed by the division of a generative element, it
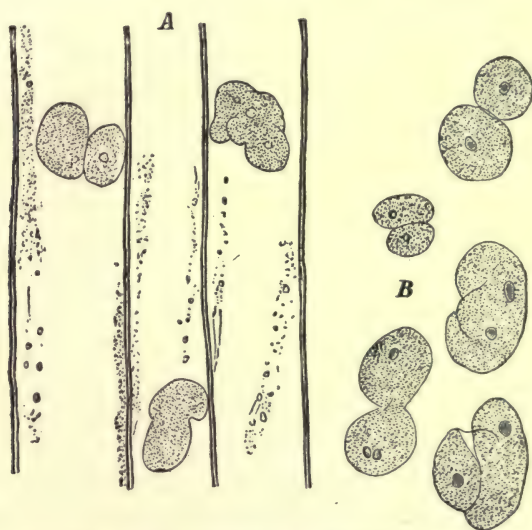
FIG. 132.   Nuclei of older internodes of *Tradescantia Virginica*, in indirect, or
amitotic division.   *A*, view of living nuclei.   *B*, nuclei after treatment with acetic
methyl-green.   After Strasburger.

may divide one or more times, but it, or its derivatives, will fol-
low a course similar to that described below.   The protoplast at
first consists of a nucleus which occupies the larger share of the
volume of the cell, and a comparatively small amount of cyto-
plasm, which is free from vacuoles.   The latter contains a large

[2] Nathansohn, A.   Physiologische Untersuchungen ueber amitotische Kerntheilung.
Jahrb. Wiss. Bot. **35** : 48.   1900.

amount of substances having a powerful attraction for water, and this brings solutions into the cell containing nutritive compounds. The pressure of the turgidity set up enlarges the cell by stretching its membranes, and the increase in volume is followed by a corresponding increase in the cytoplasm as a consequence of the rapid assimilation that ensues at this period. The nucleus may follow this increase slightly in certain specialized instances in which the final fate of the cell is that of a glandular secreting element, but in general constructive tissues the nucleus does not increase, or even maintain the size shown immediately after division. The increase of the cytoplasm continues until the cell has reached its full differentiation, or adult form. An accompanying enlargement of the vacuoles has ensued.[1] The length of life of the cell after maturity shows the greatest variation. Parenchymatous cells of the pith or cortex may remain alive and active for a long term of years and the medullary rays of the beech are known to live for more than a century in some instances. The tracheids may live two or three years, while the vessels are perhaps shorter lived. In general it may be said that the length of life of tissues, the major functions of which are mechanical, is comparatively brief.

357. Size of Cells. Fit a compound microscope with an eye-piece micrometer, that has been calibrated for the combination of lenses with which the instrument is fitted, and ascertain the exact dimensions of a number of cells in various tissues. Compare the parenchymatous cells of different organs of the same plant. Estimate the actual size of the guard cells of the stomata, and the size of the opening which they regulate. Compare root-hairs from different species.

358. Average Size and Rate of Growth of Some Unicellular Organisms. Cultivate any convenient species of *Spirogyra* in a small glass aquarium at temperatures between 8° and 22° C. Mount a few filaments on a glass slip and place on the stage of

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[1] Minot, C S. On certain phenomena of growing old. Proc. A. A. A. S. 1890. Minot, C. S. On heredity and rejuvenation. Amer. Naturalist. **30 : 1, 89.** 1896.

a microscope in the morning in a period in which rapid growth is supposed to take place. Use the eye-piece micrometer and determine the total length of a half dozen cells in a chain. Set the preparation in bright sunlight and measure again at the end of two hours. Find the increase in length and determine amount for each cell. It will be of interest to repeat the experiment, placing the preparation in a dark room at the same temperature for the same length of time. It may be possible to find cells in which division by amitosis, or mitosis is in progress. This is much better seen in staminal hairs of *Tradescantia* taken from an unopened flower bud, and mounted in a 2 per-cent. sugar solution. Buds not more than 5 mm. in length will furnish the best material and the entire process will occupy nearly two hours.[1]

359. Stages in the Mitotic Division of the Nucleus. Prepare a number of sections of the tips of roots of *Podophyllum, Allium, Zea,* or *Arisaema* by the imbedding method, staining to bring out the nuclear figures and note the character of the nuclei in the cells about to divide, and the various arrangements of the chromosomes during the process.[2]

360. Amitotic Division of Cells in Stems. Cut a number of fresh sections of the

FIG. 133. Stages in the growth of cells in the growing point of a seed-plant. *A,* cell newly formed by division. *B,* cell in which active growth has begun. *C,* cell which has attained nearly half its ultimate size. *k,* nucleus. *cy,* cytoplasm. *v,* vacuoles. Somewhat diagrammatic, $\times$ about 500. After Strasburger.

[1] Strasburger. Practical botany. 434. 1900.
[2] Strasburger. Practical botany. 434–458. 1900.
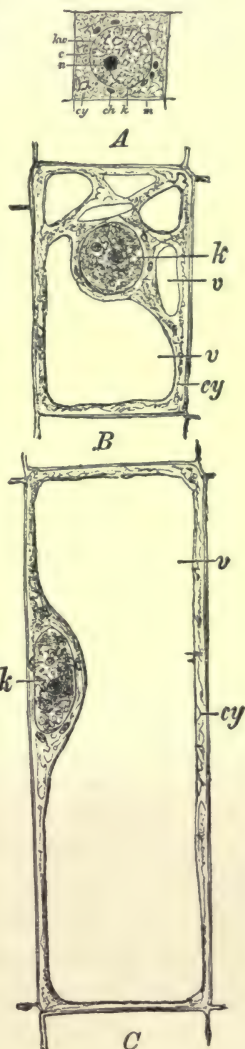
parenchymatous tissues of *Tradescantia, Cypripedium* or some other orchid and observe the character of the large nuclei. Constrictions are shown that more or less nearly divide the nucleus in parts, and this may have been repeated several times, and some of the cells may be seen to contain many nuclei, if the sections are fixed with acetic methyl-green.

Direct division of the nucleus is often induced by the presence of endophytic fungi, either as parasites or symbionts, and may be seen in the mycorhizal rhizomes of *Goodyera*.[1]

361. Course of Growth in Cells in the Apical Regions of Roots. Secure slides of longitudinal sections of root tips prepared by the imbedding method. Some should be stained with nuclear and others with cytoplasmic dyes. Cells may be seen in the successive stages of division and growth. Follow the course of the cells in the periblem cylinder. Note that an approximate cubical form is preserved as long as the cells are in the dividing stage, as indicated by the presence of mitotic figures. Measure these cells and make exact drawings with a camera lucida, showing the outlines of the nuclei of cells in the dividing zone, but which are temporarily at rest. Make similar drawings of cells which have ceased to divide. Follow the course of the nucleus several cm. from the tip in the cortex and ascertain whether it increases or decreases in size. Make estimates of the increase in size of the cell. Ascertain the amount of elongation of the cells at distances of 1 mm., 2 mm., 3 mm., 4 mm., 5 mm., etc. From the apex and from the rate of growth of the roots at hand, estimate the age, at which increase ceases (Fig. 133).

Plot a curve which would show the relative rates of elongation of the cells in the different zones denoted above.

362. Measurement of the Growth of the Apical Portion of a Root. Germinate seeds of *Zea, Pisum,* or *Phaseolus* and select a few seedlings with roots about 2 cm. in length, and lay on a piece of moistened cork. Place a thin metric scale alongside the root, and mark into intervals of 2 mm. beginning at the tip, by means of a

[1] MacDougal. Symbiosis and saprophytism. Annals of Botany. **13** : **1**. 1899.

thread held taut by means of a pair of calipers, or bow of wire. Apply the ink to the thread with a camels hair brush, and mark the root as delicately as possible. Fit a cork to a glass cylinder 6 cm. in diameter and 20 cm. in height, and fasten the seedlings to the lower side of this cork in such manner that the roots will depend vertically near enough to the sides of the cylinder, to come within the focus of a horizontal microscope. To fasten the seedlings, bore holes the size of the main axis of the seedlings in small corks, and then split the corks. Clamp them lightly around the plantlet and hold together by two short pins driven through the halves, using a third pin to fasten the whole to the stopper. After the seedlings are in place, use the horizontal microscope with ocular micrometer and measure intervals on roots exactly (Fig. 134).

FIG. 134. Demonstration of region of growth in roots. *A*, showing method of preparation of seedling. *B*, showing relative position of marks on apical portion of root at beginning of experiment. *C*, position of marks a few hours later.

Pour some water in the bottom of the cylinder and set in a dark room at a temperature of about 20° C. Measure the distances between the ink lines again in 12, 24, 48 and 72 hours, and note region of greatest growth, keeping record of the accretions. Plot curve showing the amount of growth in the regions beginning at the tip, and compare with data obtained from measurements of elongations of cortical cells.[1]

363. Growth of the Body. The multiplication of cells in generative layers, and the constant differentiation of the greater number of these to permanent tissues adds to the bulk of the framework of the organism, an increase which continues during the entire lifetime of the plant, broken of course, by the seasonal

[1] The daily periodicity and total growth of the root may be recorded by means of an apparatus described by Dr. G. E. Stone, in the Botanical Gazette, **22** : 261. 1896.

periods of rest. Additions to the volume of a plant, like additions to the volume of a cell, are also accompanied by permanent alterations in the form of the body. Unequal accretions along the various axes, and development of new members are the principal causes to which change of form may be directly ascribed.

This unequal growth is due to the localization of the generative tissues, or growing points. Additions to the body may only occur in the vicinity of growing regions, or cambium layers.

Growth of the body is not always attended by an increase in the gross weight. Thus during the earlier stages of development of a seedling, the combustion of material stored in the endosperm may be so great that the gross and dry weight decrease during the process, and the same is true of the germination of such formations as the tuber of the potato. Again, in the later stages of the life of the larger plants, the accretions from the formation of new material may not counterbalance that used in the liberation of energy, with no consideration
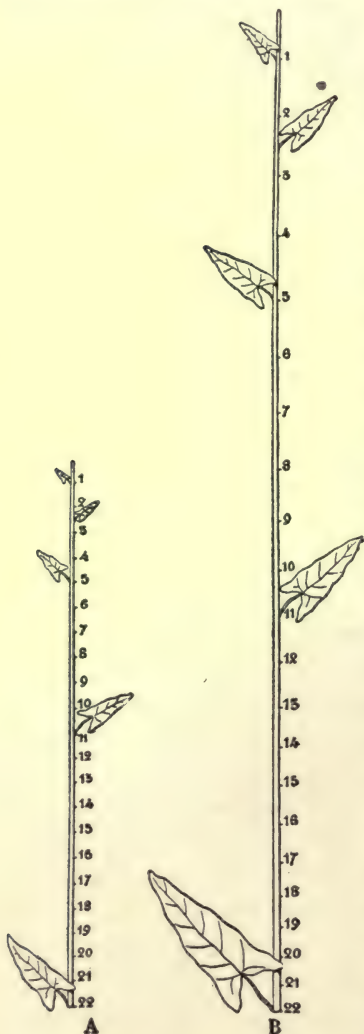
FIG. 135. Measurement of growth of the apical portion of a stem of bindweed. *A*, showing terminal portion of stem with marks of India ink 1 cm. apart. *B*, same 24 hours later in which the elongation of the various sections may be seen. The maximum rate of growth has been shown by the 5th interval from the apex (See Fig. 136). After Bonnier and Leclerc du Sablon.

of the adaptations by which the plant often cuts off large portions of its body under adverse, or seasonal, conditions.

364. Growth of Stems. Cultivate seedlings of *Phaseolus* until the stems are several cm. in height and show three or more internodes. Mark each internode into intervals of 1 cm. by means of India ink lines, and keep under good culture conditions at a temperature of 20°C. Measure the distances between the lines 24 and 48 hours later. From the data thus obtained ascertain the regions in which growth ensues, the region of greatest growth and the rates of growth in the internodes of various ages. Plot a curve illustrating these points. To do this, draw a horizontal line one-tenth of the length of the portion of the stem under observation. Divide it into millimeter intervals. Draw a vertical line from the left end representing the actual growth in length of the apical section of the stem. Draw a similar line from the next millimeter interval representing the growth of the second interval of the stem, and also the successive portions of the stem. Join the extremities of these lines and the curve produced will give a graphic representation of the growth of the stem (Fig. 136).[1]

Repeat the experiment on a larger plant, and ascertain how many internodes are growing simultaneously. Is the region of greatest growth in the same position in internodes of different ages? Describe the movements of the region of greatest growth in each internode and also the region of the greatest growth in the entire stem.

365. Growth of Petioles and Peduncles. Secure a number of rootstocks of some acaulescent plant, such as a violet. As soon as the petioles have attained a length of a few cm. mark off into intervals of a cm. by means of India ink, and measure these intervals from day to day to determine the rate of elongation of the whole organ, and the zone of maximum growth. Is the zone of maximum growth always in the same relative position? Repeat with the scape of *Arisaema*, *Narcissus* or the petiole of any convenient plant.

[1] Bonnier, and Leclerc du Sablon. Cours de Botanique. **1** : 144. **1901**.

366. Growth of a Leaf with Parallel Veins. The elongation of
a leaf, or its extension in any direction, bears a direct relation to
the arrangement of the mechanical tissues. Germinate some
bulbs of *Narcissus*, tulip, or any convenient plant and measure
intervals to determine the zone of greatest growth and the rate
of elongation, as in 364.

367. Growth of a Leaf with Netted Veins. Many leaves attain
the major part of their extension, or growth, before unfolding from
the bud, and show but little action except placing the lamina in
proper position after the bud opens. Select some species in
which the newly emerged leaf is but a fraction of the size of the
adult form, and make an index mark at the base of the lamina.
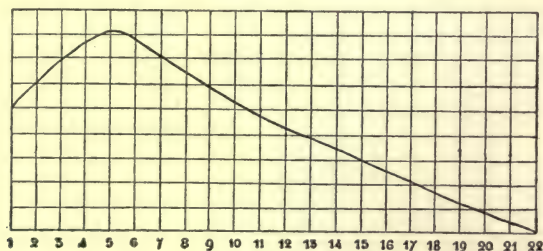
FIG. 136. Curve showing relative amount of growth in the terminal portion of a
stem of bindweed in 24 hours (see Fig. 135). The basal line is divided into the
same number of intervals as the stem, and the amount of elongation of the corre-
sponding section of the stem is measured vertically from the point at which the num-
ber is placed. The line joining these measurements forms the curve illustrating the
relative rates of growth of the regions of the apical part of the stem. After Bon-
nier and Leclerc du Sáblon.

Measure exact distance to the tip. Also place a line of dots a
cm. apart, at right angles to the main axis. Ascertain daily ex-
tension in length and breadth. Determine the regions of maxi-
mum growth in both directions.

368. Course of Growth. The growth of nearly all organs is ac-
companied by increase in volume, as well as weight. The activity
of the organ in making additions to its living substance, permanent
material, or stored food, may be followed by means of devices
for making a continuous record of the amount of elongation of

the axial or radial dimension, or of the increase in weight. Increase in thickness of most organs is due to the direct activity of the generative layer, the development, and expansion of the tissues formed, and is very minute and difficult to estimate. Such growth has been found to follow the same laws as that exhibited by the elongation of the axis.[1] Measurement of the elongation of an organ through any considerable portion of the period during which it is passing from the rudimentary stage to maturity, affords an opportunity for determination of the rhythmic action of protoplasm, and also of analyzing the influence of various factors on the process. The best method for the measurement of growth consists of the use of some instrument in which the tip of an organ, which is being pushed forward during growth, is attached to the short arm of a lever, the tip of the longer end of which, carrying a pen traces a line on a cylinder actuated by a clockwork. If the growth of the plant is as much as a centimeter daily the simple lever auxanometer shown in Fig. 137 will be found best, though many other good forms have been described and may be easily set up.[2] For the measurement of lesser increases, an instrument with a compound lever will be necessary if a proper analysis of the results is desired. Estimations of accretions in weight may be made by some form of continously registering, or recording balance.

[1] Frost, W. D.   On a new electrical auxanometer, and continuous recorder. Minn. Bot. Stud. **1** : **181**.   1894.

Golden, K. E.   An auxanometer for the registration of the growth of stems in thickness.   Bot. Gazette. **19** : **113**.   1893.

[2] Arthur, J. C.   Laboratory apparatus in vegetable physiology.   Bot. Gazette. **22** : **463**.   1896.

Barnes, C. R.   A registering auxanometer.   Bot. Gazette. **12** : **150**.   1887.

Bumpus, H. C.   A simple and inexpensive auxanometer.   Bot. Gazette. **12** : **149**. 1887.

Corbett, L. C.   A device for measuring plant growth.   W. Va. Exp. Sta. 9th Ann. Report. 236.   1896.   Also, An improved auxanometer and some of its uses. W. Va. Exp. Sta. 12th Ann. Report.   1900.

Ganong, W. F.   Some appliances for the elementary study of plant physiology. Bot. Gazette. **27** : **255**.   1899.

Stone, G. E.   Botanical appliances.   Bot. Gazette. **22** : **258**.   1895.

369. Measurement of Growth by Simple Lever Auxanometer.
Secure a rapidly growing specimen of *Narcissus, Arisaema*, or any
convenient plant with a leaf or stem that exhibits but little
nutation, and grows rapidly. Set the pot containing the plant
directly beneath the loop depending from the short arm of the lever
of the auxanometer (Fig. 137). Attach a small spring clamp to
a length of oiled silk cord, and allow the clamp to fasten upon the
tip of an organ which is emerging from the soil or bud, and about
to begin rapid elongation. Fasten the free part of the thread to
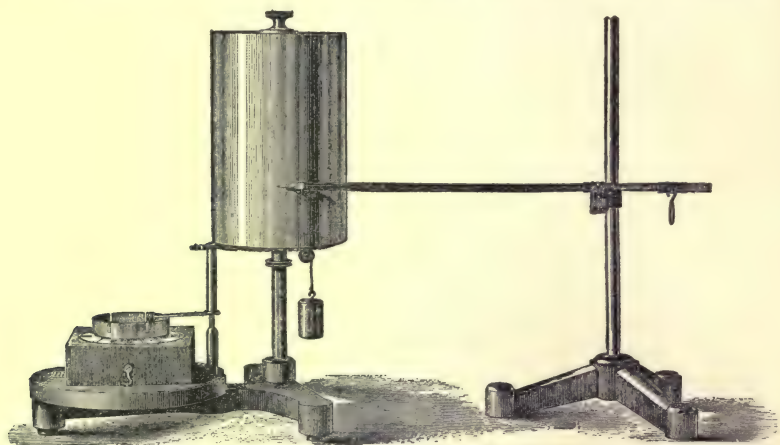
FIG. 137. Cambridge lever auxanometer. The pot containing the growing plant
is placed between the feet of the tripod support and a thread run to the loop above.
The loop, as well as the fulcrum, are attached to the lever by sleeves which may be
moved in either direction. The long arm of the lever, bearing a pen, traces a line
on the paper covering the cylinder. The clockwork to the left releases a clutch at
regular intervals and allows the suspended weight to turn the cylinder through a small
arc of revolution at regular intervals, the length of which is under control of the
operator.

the loop on the short arm of the lever at such length that the
long arm of the lever is raised and is in contact with the surface
of the cylinder near its upper end. Adjust the lever previously
so that the two arms will bear the ratio of one to six. Now
remove the cylinder from position and fasten to it a sheet of
smooth paper covering its entire surface. Hold in the smoke

of a small bit of ignited camphor until the paper is covered with an even layer of soot. Adjust in place again and set the clockwork in action, so that the cylinder will be given a partial turn every hour. The growth of the organ will allow the short arm of the lever to rise a distance equal to the amount of elongation, and the long arm to fall six times this amount, if that proportion has been established between the two arms of the lever. The gradual descent of the lever will trace a straight line in the soot on the paper showing the multiplied growth during the hour : at the end of that period the clock releases a clutch
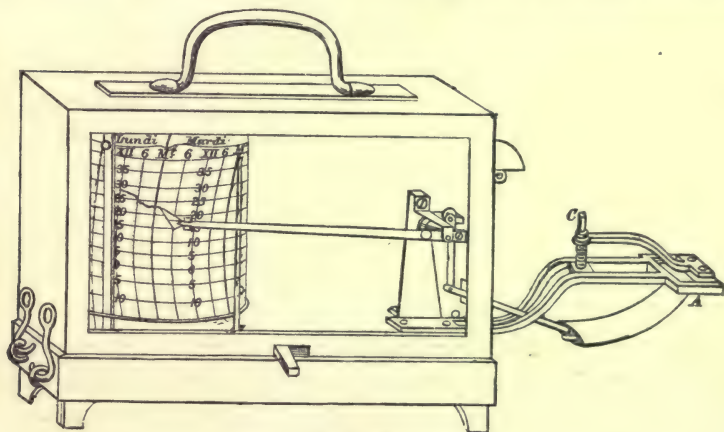
FIG. 138. Thermograph.

and allows a weight to turn the cylinder a short distance during which movement the point of the lever traces a straight horizontal line. The process is repeated every hour and when the lever has reached the lower end of the cylinder it will have made a line resembling the section of a set of stair steps, of which the vertical lines represent multiplied growth, and the horizontal lines the hours. Shorten the thread and thus raise the tip of the lever to its former level and allow it to trace another line : repeat in such manner as to secure a record for several days if possible. Set a thermograph near the instrument and secure a continuous record of the temperature also.

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Care must be taken to have the instrument on a solid stand or support, and while a proper supply of water must be given the plant, yet no disturbance of the organs attached to the instrument must be made. Careless handling of the lever may exert intense stretching pulls upon the plant which may vitiate the results for several hours, by calling out the reactions to such stimuli.

The data obtained by the auxanometric measurements should be expressed in graphic form by means of a curved line. Take the smoked paper from the cylinder, cutting it by a single vertical slit. Lay in a shallow dish of sufficient size and flood with a solution of shellac in alcohol. Hang up and allow it to dry. Secure some double ruled paper with squares of a millimeter and centimeter. Begin at the lower left hand corner of the paper and pass along the lower line of the ruled portion to the first centimeter interval. Disregard the amount of elongation shown during the first hour after the instrument was adjusted. Measure the length of the vertical line representing the second hour's growth, and measure five times its length on the vertical line at the first centimeter interval on the paper placing a dot to mark the point. Transfer the measurement of the second hour to the next line on a centimeter interval on the paper, and so on until all of the records have been placed on the ruled paper. Now connect all of the dots by a line and the resultant curve will show the relative amounts of growth at different parts of the day, and if the record is continued will embrace the grand period of growth of the organ. Transfer the record of the thermograph to the same paper and the influence of temperature upon growth may be seen directly. Care must be taken that the records of the auxanometer and thermograph for the same hour are placed one directly above the other.

370. A Precision Auxanometer and its Use. Difficulty may be encountered in securing plants that grow with sufficient rapidity to be capable of measurement by the simple lever auxanometer, in which instance the form shown in Fig. 139 will be found more useful. This type of instrument is sufficiently delicate in its ac-

tion to measure accretions in growth of no more than a millimeter weekly with reliable results, and is far steadier for all purposes than any which have been described so far, and was designed by the author to take observations upon such forms as the slow growing succulents, and plants kept for long periods in darkness. It may be kept in action for a week with no attention whatever. This instrument is furnished with a cylinder 9 cm. in diameter and 9 cm. in height which is kept in continuous motion by a clockwork
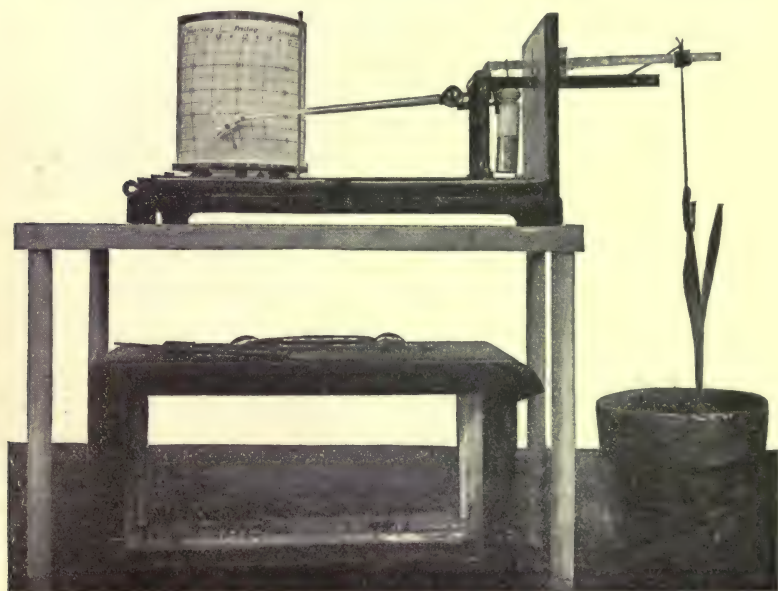
FIG. 139. Precision auxanometer attached to leaf of *Hyacinthus Belgicus*. A small metal clamp engages the tip of the leaf, and a thread attached to the clamp passes up to a sleeve attached to the outer arm of the lever. The actual amount of growth is magnified 45 times when the instrument is arranged as above. The thread has been shortened and the pen is in contact with the paper on the lower part of the cylinder as at the beginning of the experiment. The cover under the stand may be used to protect the cylinder and mechanism from dust and moisture. The pot containing the plant rests upon a plate which may be lowered or raised by the action of a screw; when the growth of the plant allows the pen to reach the top of the cylinder the plate is lowered at once to start a new tracing near the lower edge of the paper, without handling the plant or readjusting the clamp or fastenings (See Fig. 142).

over which it rests. The clockwork is attached to a cast-iron plate 13 cm. wide and 27 cm. long which also serves to support the levers. The cylinder is provided with sheets of paper of sufficient width held in place by a vertical metal strip which enters a slot in the rim attached to the lower end of the cylinder, and clamps the upper edge of the shell of the cylinder. The sheets of paper provided for this instrument are specially ruled with straight horizontal lines 1.5 mm. apart, and transversely to these with curved lines 2 mm. apart, having a radius equal to the arm of the lever which carries the pen. An aluminum lever 5 mm. in width is carried by pin-wheel bearings between two upright posts. The long arm of this lever is 15 cm. in length and carries a swinging aluminum pen which prevents any undue friction upon the paper, for which also a further regulating device is provided. The short arm of the lever may be varied in length from 5 mm. to 2 cm. and is attached to the short arm of a second lever, the free end of which projects beyond the general outline of the apparatus. The two arms of this lever have the relative lengths of one to three, and the plant may be attached to any point on the free arm by means of a loop attached to a slide. The short arm of the last lever is weighted so that the pen rests at the upper edge of the paper when not attached to a plant. When the tip of a plant is attached to the free end of the lever by means of a cord and clamp as in the previous experiment, the cord is shortened until the pen is in contact with the lower edge of the paper, or near it. The actual contact of the pen is prevented by a small rod until the instrument is properly adjusted. When all is in readiness the pen is allowed to come in contact with the paper and the extension of the plant allows the pen to rise, tracing an irregular line as shown in Fig. 142. The curved lines are such distances apart that the interval between two of them is carried past the pen in two hours. The horizontal lines being 1.5 mm. apart the amount of movement of the pen upward during the two hours may be easily read off, and plotted. The tracings being made with ink are permanent and may be filed for reference, and

as the slips of paper are of the same size as those of the thermo-
graph, the temperature curve may be transferred directly to them
or to the sheet containing the plotted curve.

371. Measurement of Growth by Weight.   Measurement of the
growth by weight may be undertaken successfully only in mas-
sive organs, in which the interchange between the organ and the
atmosphere in the form of gases and watery vapors is at a mini-
mum, and hence this method may be used with profit only in
estimating the increase of large fruits.   Cultivate some vigorous
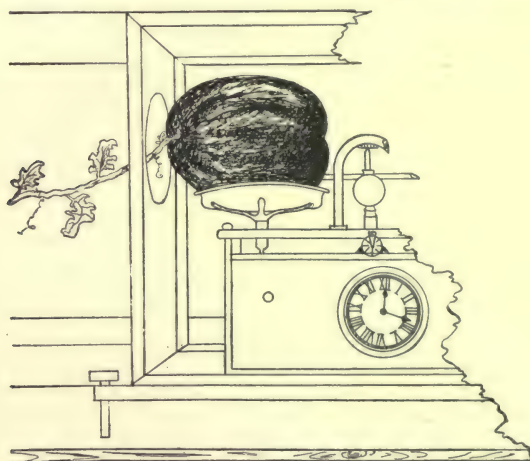
FIG. 140. Showing method of arranging a fleshy fruit on pan of Anderson auto-
matic registration balance.   Accretions in weight are equalized by weights dropped
in the opposite pan of the balance, and a record is made by a pen tracing.

variety of squash, watermelon, or any cucurbit with a large fruit,
and train the vines so that the branch bearing a young fruit may
be carried to a registering balance, or if this is not available, to
some form of weighing apparatus sensitive to half a gram, and
with a capacity of 10 kilograms.   The fruit must be adjusted so
that its full weight is carried on the scale pan, and the branch to
which it is attached bends freely to allow the action of the bal-
ance.   If a registering balance is used it will need but little atten-
tion, but should be adjusted at least once daily.   If an ordinary

balance is used, it should be adjusted by the addition of weights at least six times daily, making the adjustments as late at night and early in the morning as possible.

A thermographic record should be made, and also a continuous hygrometric registration, and both curves plotted on a sheet with the accretions in weight. At certain periods in the development
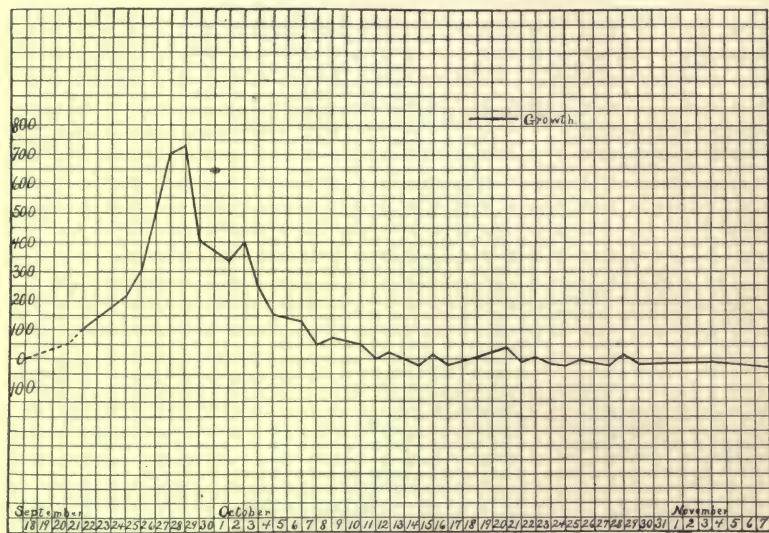
FIG. 141. Grand period of growth of *Cucurbita pepo* determined by accretions in weight. Pollination was accomplished about September 18th, and the record was begun September 21st. Continuous increase in weight ensued through a period of 20 days ending October 11th, which comprises the grand period of growth. After the latter date, irregular gain and loss was measured for 17 days, after which the loss in weight was continuous although slight. The intervals in the horizontal base line denote days, and in the vertical lines each interval denotes gain or loss of 50 grams. After Anderson.

of a large cucurbitaceous fruit it may be expected to increase as much as one gram per minute, and make a total daily increase of over 700 g.[1]

372. Periodicity of Growth. The curves plotted in the previous experiments demonstrate that the rate of growth is not

[1] Anderson, Alex. P.   The grand period of growth in a fruit of *Cucurbita pepo* determined by weight.   Minn. Bot. Stud. 1: 238. 1894-1898.

uniform at all stages of development, or at all parts of the day.
An analysis of the curves shows that the rate is slow at first, in-
creasing rapidly to a maximum and then decreasing to a minimum,
or entire cessation upon the full stature of the organ being reached,
the entire action constituting the grand period of growth of the
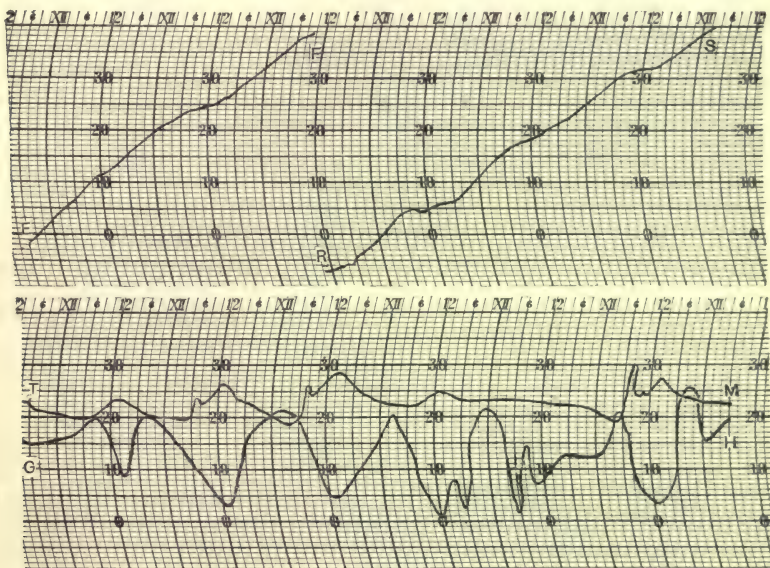
FIG. 142.   Registration, and curves of growth of leaf of *Hyacinthus Belgicus*,
showing daily variations.   The upper figure shows actual tracings of pen of precision
auxanometer (Fig. 139).   The line *E–F* is the record of growth during three nights
and two intervening days (63 hours); *R–S*, for 88 hours.   Lower figure.   *G–H*, curve
of growth showing daily variations.   The maximum occurs between midnight and 4
A. M., and the minimum about noon, at which time it is nearly zero.   A comparison
with the thermograph curve, *T–M*, shows that the minimum growth takes place at the
highest temperature, and is indicative that this temperature is above the optimum for
the species.   XII denotes midnight ; 12, noon.

organ.   If the growth of a stem is followed by actual calibration
of the elongating internodes, it will be found that the approaching
termination of the period is marked by many irregularities.
Among these, it may be mentioned, that variously distributed
regions of narrow limits continue a slight increase after the main
regions have ceased.

If the rate of growth of every hour of the day is taken into consideration it will be found that here also a periodicity is exhibited. The organ elongates but little during certain parts of the day, then begins to grow at an increased rate until a maximum is reached when it shows a lessening activity until the minimum is again shown, and in some instances a total cessation may take place ; the plant thus exhibits a daily period of growth.

The growth of a massive fruit as measured by weight shows a grand period and also a daily period. The irregularities attendant upon the attainment of mature size are most marked in such formations. In addition the cessation of growth is followed by decrease in weight due to respiration and transpiration.

The variations in weight of a fruit depend on both the growth of storage tissues and the deposition of reserve material. In this, as well as increase in volume, certain external factors such as humidity and water supply in connection with root-pressure may modify the daily rhythm, which is not lost however. Simple increase in weight does not imply growth, since a deposition of material in existing cells may cause it, and on the other hand the use or destruction of reserve material may proceed with growth. If the life of a perennial plant is taken into consideration it is to be seen that the casting of leaves in the autumn, or the annual dying away of the tender shoots above the soil exhibited by some species may greatly decrease the weight and volume of a plant.

373. Rhythm. Ever since the inception of protoplasm it has been subject to regularly recurring changes in the external seasonal conditions, and to alternating periods of daylight and darkness, with attendant changes in temperature and atmospheric moisture. This has stamped upon the vegetal organism the habit of moving in cycles which may coincide with the year, and is most marked in the flora of the temperate and arctic zones. The most noticeable feature of the cycle is the period of rest, in which the foliar apparatus is discarded and active growth is very much diminished. So deeply is this rhythm imprinted on the plant that a deciduous tree from the temperate zone will continue to shed

its leaves yearly when transplanted to the tropics, or when it is cultivated in a tropical glass house, as may be easily demonstrated with *Fagus*, *Alnus*, or *Acer*. The maintenance of approximately unchanging climatic conditions around the plant will cause some species to lose the yearly rhythm ultimately, although most kinds persist in all of its manifestations under all conditions. When the suspended conditions are again allowed to act upon the plant it resumes the rhythmic habits.

The daily rhythm is also more or less deeply implanted in the movements of protoplasm. Thus it has been seen that the nyctitropic movements persist in uniform darkness and temperature, and that the repetition of the maximum of daily growth occurs some time after the conditions which have been induced become non-operative.

374. Modification of the Grand Period of Growth. Attach a leaf of *Arisaema* to an auxanometer, and record the course of the grand period of growth, until the rate decreases to a minimum of less than a millimeter daily. Now remove the whole preparation to a dark room of the same temperature and adjust anew. Note that a second period of growth ensues which follows about the same law of acceleration and decrease as the original effort of the leaf. This shows that growth, whether developmental, or in response to certain stimuli, is rhythmical.

375. Resting Periods. Species which have become habituated to alternating periods of rest and activity acquire the power of assuming a resting condition during a certain portion of the year. and only a few species have been found which would relinquish the resting period when removed to the tropics. In some the alternation of cold and warm seasons, or wet and dry periods, has become absolutely necessary, and they may not continue existence without such changes. In others, the absence of the conditions attendant upon the resting period will result in enfeebled development. Thus, for instance, the chemical and stimulating action of low temperatures seems quite necessary to the development of many seeds and bulbs. The duration of these resting

periods may often be curtailed, and the plant brought into trophic conditions will start into activity in a healthy manner. In some cases it is necessary to intensify the principal features of the resting condition in order to secure growth at an earlier time than that of the end of the natural period. This fact is taken advantage of by gardeners in the process of forcing bulbous and tuberous species in early spring, and in consequence of this principle it is only the early blooming species which lend themselves readily to this training.

Many species, such as the lichens and mosses, find their optimum of trophic conditions during the winter, and are inactive during the summer, and each season is the time of activity of certain forms adapted to the conditions offered. It is not known whether such plants as the mosses would relinquish their resting period if brought into uniform low temperatures and moisture, or not.

376. Forcing. The exposure of seeds and bulbs, tubers and rootstocks to periods of a few weeks of low temperatures and then their cultivation in proper temperatures may result in a development as much as a hundred days earlier than might be attained under natural conditions. As a result of recent investigations it is found that subjecting the resting plants to the action of an anaesthetic may reduce still further the duration of the resting period, although such treatment, and indeed nearly all forcing exerts an ultimate depressing or exhausting effect upon the plant.[1]

377. Influence of Temperatures Upon Resting Period. Secure a lot of plants embracing seeds of oaks, hickories, herbaceous perennials and bulbs, and tubers of such plants as *Arisaema, Trillium, Convolvulus* and divide into two portions. Place one in a greenhouse room at 20° to 25° C. and the other in a refrigerator at about 8–10° C. This should be done at the end of October. Ten weeks later set all the material in moist soil, under proper

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[1] Johannsen, W. The forcing of plants by ether. Translation in American Gardening. **21** : 358. **1900**.

Bailey, L. H. Cyclopedia of Amer. Horticulture. **2** : 595. **1900**.

conditions for germination, and note the comparative periods of rest shown by the specimens kept warm, and those given an exposure to low temperatures. The specimens placed in the warm room should be packed in damp moss to prevent desiccation.

378. Conditions Affecting Growth. Temperature, electricity, food supply, free oxygen, moisture and turgidity of the plant, barometric pressure, mechanical shock, traction and compression affect the rate of growth. So far as the trophic factors are concerned it is to be said that there is a certain optimum intensity at which growth proceeds most rapidly, and any deviation from this will check increase in the body of the plant. Light has hitherto been regarded as exercising a retarding or paratonic effect upon growth, but as the result of recent investigations it is known to influence growth only indirectly by its effect upon the food-forming processes, and upon other kinds of metabolism. A number of instances are proven in which light accelerates growth, but the probability is not excluded that growth of some species of special habit may be retarded by the action of light (Chapter VIII.).

The temperature at which growth proceeds most rapidly varies with the species (Chapter VI.). Infrequent electric currents increase the total amount of growth (Chapter VII.). A proper food supply, either as a reserve or available in the substratum, must be available to form constructive material for growth. Hydrostatic pressure or turgidity is necessary for the growth of almost all cells, although a few instances are known in which increase in volume may be carried through a wide range without this force. Mechanical stimuli of all kinds decrease the rate of growth when first applied, but later the rate of growth may exceed that of the untreated specimen or in some instances fall below it (Chapter II.).

Growth implies more or less destructive metabolism and the liberation of energy, which is chiefly accomplished by means of combination with free oxygen, and this condition is necessary for all organisms except the anaërobes which use other forms of respiration. Variations caused by alterations in atmospheric

pressure are due to a complication of causes, among which may be mentioned the change in the pressure of the free oxygen, and turgidity, and unusual conditions offered the transpiratory mechanism. The most recent investigations on this subject by Schaible bring the following conclusions : The growth of shoots is accelerated, and germination is retarded by diminished barometric pressure. The retardation of germination is due to the diminished pressure of free oxygen.[1]

379. Influence of Temperature upon Rate of Growth. Fasten the cord of an auxanometer to the tip of a leaf of *Narcissus*, and record the rate of growth during two days and note whether the rate is increasing or decreasing. Now cover the plant with a bell-jar with a tubulure at the top, through which the auxanometer cord may be passed. Place several cylinders filled with crushed ice and salt in the bell-jar, to lower the temperature, and suspend a thermometer near the organ to which the auxanometer is attached. Note temperature at intervals of 30 minutes for 4 hours. Allow the temperature of the air in the bell-jar to rise as the ice melts. On the following day plot the curve of growth for the two days previous to the exposure to low temperature, and compare with the curve of the rate made in the cold. How long was necessary for the effects of the low temperature to be shown by the plant ? Note acceleration of growth as the temperature rises.

380. Age, Senescence and Death. The duration of life of a single individual plant may include but a few hours in the case of the simpler forms, or may extend over many centuries in the case of woody trees and shrubs. A cell constituting an individual bacterium may develop through a period of fifteen minutes or more, then undergo division into two or more separate organisms terminating the career of the single individual, but not by the death of living matter. The increasing complexity of the higher organ-

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[1] Schaible, F. Physiologische Experimente ueber das Wachstum und die Keimung einiger Pflanze unter verminderten Luftdruck. Beitr. z. Wiss. Bot. **4:** 94. **1900.**

Curtis, C. C. Turgidity in mycelia. Bull. Torr. Bot. Club. **27 :** 1–13. **1900.**

Galloway, T. W. Studies on the cause of the accelerating effect of heat on growth. American Naturalist. **34 :** 949. **1900.**

isms entails a course of life which embraces a senescent, or developmental period, and then a series of deteriorations resulting in death, while the species is preserved by the activity of certain rejuvenated portions of the body, which are specialized and cut off during the period of activity. Many species of herbaceous plants start from the seed, develop a shoot and form seeds, dying in less than a hundred days from the time of germination. Others develop two or more seasons before the capacity of forming seeds is exhibited, and then make seeds one or many seasons, until deterioration begins.

Plants which live many seasons add numbers of mechanical elements to their skeletons every year. The cells formed by the generative layers, and which pass into permanent form, undergo varying periods of senescence, and of endurance (356).

The senescent changes in simple organisms, like *Spirogyra* and other filamentous or unicellular organisms, are not well known, and no systematic study of this phase of plant life has been made in recent years. The cells soon reach an ultimate size in these plants, and then carry on the normal functions for a time when they begin to deteriorate. The duration of life of a higher plant is so largely influenced by external conditions that it is difficult to distinguish between phenomena of post-maturity, and those due to lack of nutrition, harmful transpiration, parasites, ravages of climate, etc. It may be easily seen upon theoretical grounds however, that the mechanism of any plant is sufficient only to serve its needs until a certain size is attained, and as a plant is constantly increasing in size its age limit is also a limit of size. Thus, for instance, a tree may grow only so long as its trunk will support the constantly increasing crown. The difficulty of supporting this crown will also be augmented in many instances by air-currents. Then again, it is quite possible for a plant to exhaust the food elements in the soil around the base of its stem, and it must drive its rootlets a constantly increasing distance through the substratum until the difficulty of transport of the soil salts permits only an insufficient supply to reach the crown.

381. Length of Life of an Annual. Ascertain the time necessary for some rapidly developing annual to attain full stature, form and cast off its seeds, and die.

382. Period Necessary for Maturity of the Cells of a Stem. Cut down a tree at least 40 cm. in diameter and test the medullary rays to ascertain to what age these elements survive. Also test the tracheids and find if they live beyond the season in which they are formed.

383. Senescence and Death in an Annual Plant. Examine any convenient herbaceous annual, and note the first stages in the steps leading to its death. In what organs are the phenomena first visible ? Cut sections of the dying stems and leaves and note changes in color of the walls and constitution of the living matter. Note the relation of the death of the plant, and the maturity and dissemination of the seeds. What organs survive longest ?

384. Death of a Perennial. Select a tree of any convenient species in a forest and ascertain the conditions which have caused its death. Note the presence or absence of parasitic fungi, and determine whether these have gained a foothold in decaying tissues, or have attacked sound wood or living tissues. Examine the tree for damage caused by animals. What portion of the tree perished first ?

385. Correlations in Growth. A multicellular plant body with differentiated tissues presents a diversity of complementary functions, which are involved in a series of mutual interactions of the most complex character. The modification of any function, or changes in the organ, in which the function is carried on, produces a disturbance in all of the other organs of the plant constituting an effort toward readjustment to the altered conditions. All of the activities of the organism are correlated, but it will be most convenient to study the results of this feature in the organization of the plant in growth. The development of a fruit as a result of the pollination of the pistil, the development of a lateral branch to take the place of a main stem which has been removed (130). and the formation of the missing organs from a stem, root, or leaf

cutting are most marked examples of the effort of a plant to re-adjust itself to changes by means of its correlating mechanism. The polarity of plants, by which one end of the axis is constituted the apex of the shoot, and the other the root-bearing end, is also a correlation by which the apical end shows a tendency to bear leaves and give rise to branches while the other gives rise to absorbing organs. This polarity is resident even in fragments of a plant, and roots in certain instances tend to form shoots from the original upper end, and new roots from the end farthest away from the original stem apex. This polarity not only extends to the longitudinal organization of the axis of the plant, but also to the radial arrangement of its organs.[1]

386. Development of Latent Organs as a Result of Correlative Stimulation. Cultivate a number of seedlings of any common herbaceous form and when a few cm. in height cut off the epicotyl just above the cotyledons. The buds in the axils of the cotyledons will be awakened and begin development.

387. Changes Induced in Flower Stalks by Fertilization. Cultivate a number of specimens of *Arisaema*, *Cucurbita*, or other convenient plant. As soon as the flower buds open, remove stamens and enclose in paper bags to prevent pollination. Secure the transference of pollen to the stigmas of others left open. Note the consequent difference in the development of the ovarial tissues and the scapes or peduncles. It will be of interest also to enclose a third lot in paper bags, and to irritate the stigmatic surfaces by rubbing lightly with a soft piece of wood, or applying 1 per-cent. solutions of various salts, such as magnesium chloride, or potassium nitrate and note results.

If *Arisaema* is used in the above experiment a specimen may be enclosed in a paper bag and pollen applied with a camel's hair brush to some of the pistils only, which will allow the growth of pollinated and unpollinated ovaries to be compared.[2]

[1] Vöchting, H. Ueber Organbildung im Pflanzenreiche. 1 : 1878.
Reinke, J. Die Abhängigkeit der Blattentwickelung von der Bewurzelung. Ber. Deut. Bot. Ges. 2 : 376. 1884.
[2] Goebel, K. Organography. 1 : 269–270. 1900.

388. Correlative Changes in Growth Due to Injuries. It is well known that the destruction of one of the branches of a shoot is followed by the accelerated development of other contiguous branches for the purpose of taking up the functions of the lost member. It may also be demonstrated by the following experiment that an injury to an organ of the shoot is followed by responses in the most distant parts of the body.

Germinate a number of seedlings of *Pisum* or *Phaseolus* until the roots are about 2 cm. in length. Select a dozen of equal development and make a thin mark with India ink near the base of the root, and measure the distance exactly to the tips, keeping the records identified with every specimen. Fix the seedlings in small cylinders filled with water, by means of perforated cork stoppers, allowing the roots to be submerged. Remove the shoots from half of the plants by means of a sharp knife, cutting cleanly across immediately above the cotyledons. Set all of the preparations in a moist chamber consisting of a large bell-jar at a temperature of 20–22° C. Measure the lengths of the roots 24, 48, 72, and 120 hours later. Ascertain the difference in the amount of growth shown by the roots of the normal and decapitated plants. Follow the growth of the plants for a week and find what length of time is necessary to equalize the rate of elongation of the roots and shoots in both series.

Repeat the experiment, but cut away the roots instead of the shoots and note rate of growth of the shoots in the normal and mutilated specimens. Special precautions must be taken to prevent desiccation of the latter by the use of cotton wool moistened with water.[1]

389. Movements Due to Correlations in Growth. The general position of every organ relative to the main axis, and to other branches is assumed in response to correlative changes in growth. Such positions are not identical in all stages of development. In

[1] Townsend, C. O. The correlation of growth under the influence of injuries. Annals of Botany. **11** : 509. 1897.

See also, Hering, F. Ueber Wachstumscorrelation in Folge mechanischer Hemmung des Wachsens. Jahrb. Wiss. Bot. **29** : **132.** 1896.

fact the correlations demand changes in position or organs in different stages of the development of the axis, or of the same organ under different external conditions. Thus it is to be seen that leaves unfolding from the bud may hold a vertical position at first, which is changed to a horizontal position during functional activity, when they are also subject to the directive influence of external agents such as light and gravity. Leaves formed later may retain their vertical position and form a protective covering from the growing point of the stem to which they are appressed.

Such correlations prevent the interlocking and entanglement of the various organs, and allow their anisotropic positions, in response to the external directive factors, to be taken with least effort.

It is also probable that correlations of this kind are active in regulating the movements of organs of plants placed in contiguity. Recent results obtained by F. G. Smith show that the plant does not allow its branches to be irregularly entangled with those of neighboring individuals.[1]

Movements of correlation are most marked in dorsiventral organs, and are caused by the unequal growth of the opposite flanks ; the excess of growth on the upper side of an organ being termed epinasty and on the lower side hyponasty.

390. Epinasty and Hyponasty. Observe the form of fronds of any fern that have just emerged from the soil, and note the coiled position of the apical portion. The fronds are seen to be hyponastic and then epinastic. Make similar observations on seedlings of *Allium* or any cucurbitaceous plant, noting the changes in the growth of the two sides. The form assumed in response to epinasty or hyponasty in these instances is for the purpose of penetrating the soil without damage to the more delicate portions of the plant. Modifications of the positions taken may be induced by the cultivation of the seedlings in darkness, setting up new and different stimuli.

[1] Smith, F. G. A peculiar case of contact irritability. Bull. Torr. Bot. Club. **27 : 190. 1900.**

Take some vigorously growing specimens of *Taraxacum* from the soil and wrap the roots with wet sphagnum. Support in an upright position. Note positions assumed by the leaves. Invert and make same observations. Are the positions of the leaves due to epinasty or hyponasty, or to geotropism ?[1]

391. Carpotropic and Gametropic Movements. A large number of movements are carried on by the primary or accessory reproductive organs for the purpose of promoting fertilization, dissemination of seeds or spores or protection from climatic elements. These movements may be directed by external stimuli, principally that of gravity, or may be epinastic or hyponastic. Movements of this character entail an accession of sensibility to the external stimulus at a certain stage of development, or a change of form of reaction to this stimulus. Thus a petiole may be apogeotropic until fertilization or pollination is accomplished, when it may become progeotropic. Similar irritability to light is found, and instances are not lacking in which a second change is made. The stimuli by which auto-carpotropic movements are set up, are released by developmental changes. Opening and closing of calices, movements of stamens and pistils,
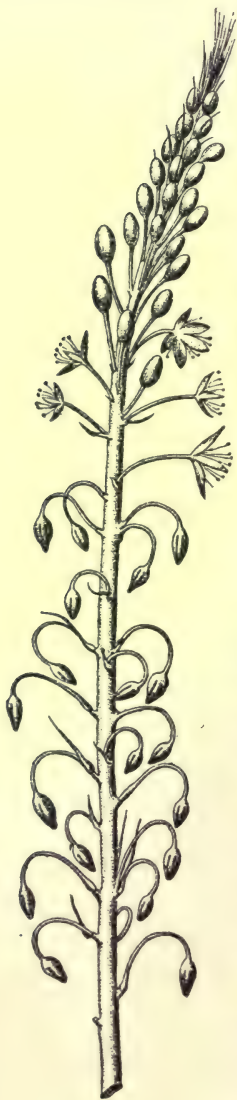
FIG. 143. Inflorescence of *Bulbine longiscapa* showing carpotropic movements. The unopened flower buds stand approximately erect, appressed to the axis : the pedicels of open flowers are horizontal and the pedicels curve downward after the seed-capsules begin to develop. After Hansgirg.

[1] Day, R. N. The forces determining the position of dorsiventral leaves. Minn. Bot. Stud. **1** :743. 1894–1898.

and curvatures of petioles of some species are examples of this action.

392. Carpotropic and Gamotropic Movements of Peduncles and Other Organs.   Cultivate a number of individuals of any species of *Fragaria*, or any member of the poppy family and note the positions assumed by the flower bud, open flower and developing fruit.   Determine position of motor zone producing movement.

FIG. 144.   Carpotropic movements of *Allium Neapolitanum*.   *D*, inflorescence emerging from bracts, in which stage the scape is curved with its apex directed downward.   *C*, the scape is straightening, and one of the pedicels, which has emerged from the bracts, is seen to be apogeotropic.   *B*, flowers all open, with pedicels in positions assumed in response to growth or correlation stimuli, which is further modified in the final position in *A*.

Follow the movements and positions of the calyx.   Make similar observations on *Tradescantia* or *Hippeastrum*.   Use the clinostat and dark room and determine to what forces each movement owes its stimulus (131).

393. Carpotropic Movements of Aquatics.   Follow the movements of the scapes of *Pontederia, Vallisneria*, or of almost any aquatic plant and note the positions assumed.[1]

[1] Hansgirg, A. H.   Physiologische und Phycophytologische Untersuchungen.   1893.

Hansgirg, A.   Neue Untersuchungen ueber den Gamo- und Karpotropismus, sowie ueber die Reiz- und Schlafbewegungen der Blüthen und Laubblätter.   Prague.   1896.

Hansgirg, A.   Berträge zur Kenntniss der Blüthenombrophobie.   Prague.   1896.

XIV. REPRODUCTION

394. Origin of New Individuals. The primal purpose of every individual is to give rise to others, thus ensuring the continuation of the species. The differentiation and separation of masses of protoplasm from the body, which undergo rejuvenescence, and then pass through the chief stages in the development of a typical individual of the same, or alternate generation, constitutes reproduction.

New individuals may arise by two general methods, according to the character and origin of the protoplasm from which they develop, which may be distinguished as monogenetic, vegetative or asexual, and digenetic, or sexual methods of reproduction.

Vegetative reproduction is that method by which a single mass of protoplasm consisting of one or more cells is cut off from the parent and produces a new individual. This method gives rise to a series of individuals perpetuating the qualities of a single line of ancestors, which may become more or less fixed and accentuated in successive generations. Vegetative reproduction is carried on by plants of nearly all of the families in the vegetable kingdom, and is the only method known in some forms.

Two kinds of vegetative reproduction may be distinguished, according to the nature of the special bodies concerned : somatic propagation, budding or gemmation, and spore reproduction. In somatic reproduction a mass of cells is cut off from the parent and undergoes development into a new individual. A wide variation is shown however. Gemmae may consist of but one cell in some species, while in others the reproductive body is one of the organs of the plant, but little differentiated from its vegetative form. Spores are generally single protoplasts of specialized origin capable of giving rise to a new individual, and in this con-

nection it is to be said that many plants, especially fungi, produce bodies termed spores that are multicellular.

Sexual reproduction is the method by which two masses of protoplasm, gametes, of unlike physiological character and generally showing morphological distinctions, are fused to form a single cell, or spore, capable of giving rise to a new individual. The gametes are usually directed to each other by chemotaxis, and the mechanism of their union is most diverse in various species. Neither of the gametes are usually capable of developing into an individual alone. The union of two gametes in sexual reproduction brings together the multi-complex inherited qualities of two parents with their similarly multi-complex ancestry, and tends to obliterate the isolated variations shown by either parent.

The organs concerned in both asexual and sexual formation of spores generally show such highly differentiated morphological structure, and diversity of development that the study of their activity constitutes a separate branch of the subject, and lies beyond the limits of this volume. The following discussions and experiments will therefore deal only with the forms of asexual reproduction which might be included in somatic processes, with one or two examples of the factors operative in calling out the activities of other mechanisms.

395. Multiplication of Individuals as a Result of Senescence and Death of a Part of the Body of the Plant. The simplest manner in which new individuals may arise among the higher plants is that by which the older parts of the main axis die away, and the separated members continue their growth, replacing the organs of which they have been deprived by the death of the older member. The separated portions may or may not take on special form or structure, a matter dependent upon the seasonal conditions which they must endure.

396. Division of Individuals in Marchantia, Azolla, Marsilea, and Lycopodium. Cultivate a number of specimens of the plants named, and note that the death of the older portions separates the branches, and that these in turn subdivide and multiply the

number of individuals in the same manner.   Cut apart the sepa-
rate branches of a thallus of *Marchantia*, and place the fragments
on a layer of moist sand in a dish, covered with a glass plate to
form a moist chamber.   Divide another plant into segments and
test the capacity of those from different parts of the thallus to
reproduce the entire individual.   If antheridial or archegonal
branches are present treat these in the same manner.[1]

397. Propagation by Gemmae and Other Special Bodies. Uni-
cellular plants carry on multiplication by simple division and
fission, the simple cell becoming an adult individual before or
shortly after it is cut off.   Species of greater complexity of struc-
ture separate masses of protoplasm for the purpose of effecting
reproduction, which in some instances differ but little from unicel-
lular spores.   Such gemmae, or brood-bodies, may consist of
chains, plates, or globular cell masses containing a few, or hun-
dreds of cells.   Gemmae are generally produced from the exter-
nal layers of the body, although instances are not wanting in
which they develop in the internal tissues.   The formation of
gemmae may sometimes be induced by mutilations, and their
appearance is generally due to modifications in the external tro-
phic factors.

Propagative bodies among the mosses are generally modified
leaves, stems, or antheridial branches, while the same purpose is
effected in the ferns, fern allies, and seed plants by buds devel-
oped in diverse ways.   These may be considered with reference
to the part of the body from which they originate.[2]

398. Reproduction by Gemmae of Georgia (Tetraphis) pellucida.
Take up some decaying wood on which is growing *Georgia pel-
lucida*, in autumn or spring, and remove to a moist chamber in the
laboratory.   Examine the cup-shaped receptacles at the ends of
the branches for gemmae.   These are irregular, lens-shaped
bodies borne on stalks in place of antheridia among the para-

[1] Vöchting, H.   Ueber Regeneration der Marchantieen.   Jahrb. Wiss. Bot. **16** :
367.   1885.

[2] Goebel, K.   New formation of organs in regeneration.   Organography of plants.
Part I., p. 44.   1900.

physes. The gemmae are two or three layers in thickness in the central region and one at the margin. Two to eight cells in the portions near the margin are seen to be thin-walled with the outer membranes convex, constituting the *nematogones*, or cells active in propagation. Although gemmae are produced throughout the year yet none may be found on the material collected. In this case allow the moss to grow for a few weeks in the moist chamber and examine again. When found detach a number, and

FIG. 145. Gemmae of various Muscineae. 1, *Marchantia polymorpha* with cups containing gemma. 2, longitudinal section through cup of *Marchantia*. 3, gemma of *Marchantia*. 4, *Tetraphis pellucida*. 5, stem of *Tetraphis* bearing a cup containing gemmae. 6, longitudinal section of cup of *Tetraphis* showing gemmae. 7, 8, single gemmae of *Tetraphis*. 9, stem of *Sciuroides* with brood-bodies. 10, single brood-body of *Leucodon*. 11, development of brood-body on rhizoids thrown out by a leaf of *Campylopus fragilis*. 12, 13, 14, stages in the development of gemmae from apex of leaf of *Syrrhopoden scaber*. 15, *Aulacomnion androgynum*. 16, stalk of *Aulacomnion* bearing brood-bodies. 17, 18, single gemmae of *Aulacomnion*. After Kerner.

place in moist sand in a small dish and cover with a bell-glass. Follow the germination of the nematogenous cells, and note the development of the protonema, and the production of a new gametophyte.

399. Propagation by Modified Leaves in Aulacomnion. Collect specimens of *Aulacomnion palustre*, or *A. androgynum*, which may usually be found on charred logs, stumps, or wet boggy soil, and note that the leaves on the upper, terminal part of the stems are of different form from those below, and are easily detached. Examine with low power and make out general structure. Place a number of the thickened leaves in a moist chamber and examine three days later. Note the number and location of the nematogenous cells and their germination. Follow the course of growth of the protonemae and the appearance of the gametophyte.[1]

400. Gemmae of Scapania. Collect specimens of *Scapania nemorosa* from its habitat on moist banks or rocks and find the unicellular gemmae on the tips of the upper lobes of its leaves and the apex of the stems. Remove and cultivate in moist chamber. Note the development of the new gametophyte, and compare with that of the germination of the gemmae of the mosses.

401. Gemmae of Kantia. Collect specimens of *Kantia trichomanis* from moist banks, ditches or decaying logs and note the clustered gemmae found on the tips of the orthotropous shoots. Place in moist chamber and follow development. Compare with that of *Scapania*.

402. Gemmae of Marchantia and Lunularia. Examine the upper surfaces of thalli of *Marchantia*, or *Lunularia* until receptacles are found containing numbers of small green globose bodies constituting gemmae. Cut a cross section of the thallus through the receptacle to obtain a view of the short stalks on which the

[1] Correns, C.   Untersuchungen ueber die Vermehrung der Laubmoose. **191**, 206. 1899.

Goebel, K.   Organographie der Pflanzen.   Part 2, Hft. **1**. 273.   1898.

Heald, F.   A study of regeneration as exhibited by mosses.   Bot. Gazette. **26** : 169.   1888.

gemmae are borne, and also find these bodies in the earlier stages of development. Place a number on moist sand in a bell-jar and observe the germinating action. At what special points may growth set up? Note the character of the reserve material stored in the gemmae.

Carefully divide a gemma in halves longitudinally by means of a sharp razor, and a second, transversely and place the halves in a moist chamber. Are new individuals formed from the segments?

403. Bulblets of Filix (Cystopteris). Collect a few leaves of *Filix* (*Cystopteris*) *bulbifera* bearing bulblets on the lower surfaces of the midribs. Dissect and note structure of the bulblets: They will be found to consist of a number of thickened scales attached to a short stem. Place a few of the buds on moist sand under a bell-jar. From what parts are

FIG. 146. Showing bulblets of *Filix bulbifera*, with portion of leaf on which it is borne, and germination of same. After Atkinson.

new plants formed? Does the bulblet itself enter into the new plant? Determine the character of the storage material.

Dissect a few bulblets and place the separated scales and stems in the moist chamber, some with the inner and others with the outer surface uppermost. Ascertain the regions of the scales capable of giving rise to a new plant. Cultivate a number of bulblets in darkness. Test the endurance of the bulblets to desiccation by allowing several to be exposed to the air in an ordinary room for several days or weeks.[1]

[1] Rostowzew, S. Die Entwickelungsgeschichte und die Keimung der Adventivknospen bei *Cystopteris bulbifera* Bernh. Ber. Deut. Bot. Ges. Gen. Versammlungs. Hft. **12**: 45. 1894.

The bulblets will be found to be extremely resistant to variations in temperature and moisture.[1]

404. Adventitious Buds of Asplenium bulbiferum. Observe a number of specimens of *Asplenium bulbiferum* in a greenhouse and note the numerous buds arising at various places on the margins of the leaves. Select a portion entirely free from buds and lay on moist sand to ascertain if the separated leaf is capable of giving rise to the buds or new plants. Dissect a bud and place the separated scales in a proper culture chamber. Are the leaves capable of reproducing the plant? Test the endurance of buds to desiccation, and to temperature of dry and moist air.

405. Adventitious Buds of Polystichum angulare. Examine vigorous individuals of *Polystichum angulare*, growing in a green-house, for the adventitious buds which arise in the axils of the pinnae. Dissect and note structure. Germinate in moist chamber, and observe from what scales and
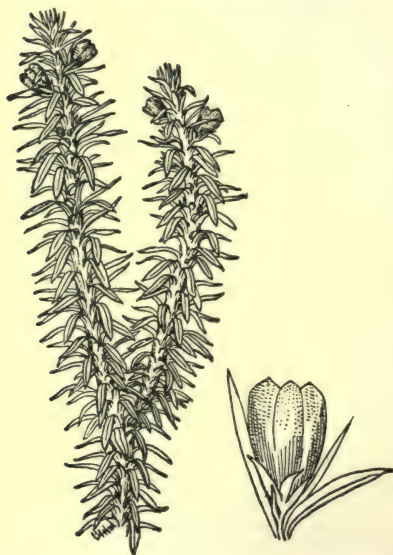
FIG. 147. Brood-bodies of *Lycopodium lucidulum*. After Atkinson.

what regions the new shoots arise. Place the separate scales on moist sand under a bell-glass and determine their capacity for propagation. Identify the storage material present. Place a portion of the leaf in a moist chamber and find if it is capable of giving rise to a new plant. Other species of *Asplenium* form similar

[1] Heinricher, E. Ueber die Wiederstandsfähigkeit der Adventivknospen von *Cystopteris bulbifera* Bernh. gegen das Auftrocknen. Ber. Deut. Bot. Ges. **14**: 234. 1896.

Heinricher, E. Nachträge zu meiner Studie ueber die Regenerationsfähigkeit der *Cystopteris* Arten. Ber. Deut. Bot. Ges. **18**: 109. 1900.

buds, and may be used in the experiments. Another fern, *Ceratopteris thalictroides*, often cultivated, offers interesting material for these tests.

406. Propagation of Lycopodium. Examine vigorous specimens of *Lycopodium lucidulum* for the wedge-shaped or heart-shaped bodies to be found in the axils near the apices of the stems. Place a number in a moist chamber and note the manner of their germination. Cut into halves by longitudinal and transverse incisions, and ascertain the location of the growing points. Test endurance to desiccation, and determine character of storage material [1] (Fig. 147).

407. Vegetative Reproduction by Means of Buds Among the Seed Plants. The development of new individuals from buds formed on various portions of the bodies of higher plants is exhibited in great variety. It will

FIG. 148. Development of propagative buds of *Asplenium bulbiferum*. After Atkinson.

be convenient to discuss some of the principal types according to the member from which the propagating body arises.

408. Origin of New Plants from Roots. The roots of a large number of plants are capable of forming buds which reproduce the individual. This capacity is shared by *Botrychium*, *Ophioglossum* and perhaps other ferns. Buds are generally developed on old roots in which decortication has occurred, and secondary thickening has taken place, and those forms which have been specialized for this purpose by the deposition of a large amount

[1] Sterns, E. E. The bulblets of *Lycopodium lucidulum*. Bull. Torr. Bot. Club. **15**: 317. 1888.

of reserve food.   Not all tuberous roots are capable of propaga-
tion however.   Shoots originating in roots may be seen in old
specimens of *Rubus, Ailanthus, Fagus, Crataegus, Syringa, Rosa,
Maclura, Liriodendron*, also in the thickened roots of the Convol-
vulaceae, Ericaceae, and others.

409. Cuttings from Roots.   Cut sections several cm. in length
from roots of old plants of *Rosa, Populus*, or *Rumex*, or from the
small lateral roots of horse radish, and imbed in moist sand and
keep under proper cultural conditions.   Ascertain manner and
place of formation of buds.[1]

410. Propagation by Tuberous Roots.   Secure a number of sound
sweet potatoes which have been kept in a cool dry place after
taking from the soil in the autumn, and place a few in moist sand
under proper cultural conditions.   Cut one or two others into
segments by longitudinal and transverse incisions, and place the
pieces with the entire tubers and ascertain what regions are cap-
able of giving rise to buds.   Clean a tuber carefully in water and
examine the superficial layers to determine the presence of latent
buds or growing areas in the ungerminated tuber.   Identify the
substances stored in the roots.   Note manner of translocation to
young plant.   Are juvenile forms of leaves developed by the
plants arising in this manner?   The above experiments should
be carried on at temperatures of 18 to 22° C.

411. Propagation by Stems.   Buds on stems which undergo a
special development for the purpose of giving rise to a new indi-
vidual may or may not be supplied with reserve food, and may
or may not be separated from the parent plant before its death or
maturity.   Such buds may be borne on underground branches
arising from the bases of the main stem, and such branches may
be developed to bear several buds, which like the potato are cap-
able of giving rise to many new individuals.   In other instances
propagative buds arise from trailing or decumbent branches,
which place the young plant some distance from the parent, and
thus accomplish the incidental purpose of dissemination.   The

[1] Bailey, L. H.   Nursery book, 61.   1896.

buds in the aërial axils, and also the flower buds, may undergo transformations that enable them to undergo rejuvenescence and give rise to new individuals ; a capacity exhibited by ordinary buds of an enormous number of plants, when severed from the plant by artificial methods and given proper cultural conditions.

412. Bulbs of Narcissus. Dissect a number of bulbs of *Narcissus*. These structures will be found to be simply buds with a short stem sheathed with thickened scales. Branches of the stem may be seen, bearing small bulbs of similar structure. Germinate one of the bulbs. Place the dissected parts of a second in the moist chamber and ascertain what parts are capable of giving rise to new plants. Identify the storage material. What is the fate of the stem and scales in reproduction by the germination of the entire bulb ? Repeat the above tests with any member of the lily family, or *Bicuculla* (*Dicentra*).

413. Propagation of Arisaema by Buds. Examine a number of corms of *Arisaema* in the autumn or early spring. Each corm will be found to consist of a thickened stem consisting of a few compressed internodes, the terminal one bearing a single bud sheathed by a prophyll consisting of a scale with its edges united to form a conical sheathing cover. Smaller buds may be seen at various points on the upper margin of the corm marking the position of internodes. Some of these lateral buds may have developed a small corm which becomes detached from the parent at the close of the first season of its growth, and which reproduces the entire plant. Germinate some of these lateral buds, or those derived from *A. Dracontium*, and it may be seen that the new individual does not attain the adult form and flower until the second or third year. The leaves produced the first year are of the juvenile form and resemble those of the seedling of the second year's growth. Observe the action of any arum in the germination of the corms, and repeat the above tests with *Gladiolus*.

414. Propagation of Solanum by Tubers. Place a number of potatoes in a moist chamber and note the development of buds near the apical end of the tuber. Cut these out with a sharp pointed

knife and note the growth of the nearest buds below. Repeat until all of the buds have shown signs of activity. Cut a tuber into such segments that each shall contain a bud, and imbed in

FIG. 149. Runner of a strawberry plant, developing plantlet at first internode. After Beal.

moist sand. Compare the leaves of the new individual with the forms exhibited by seedlings. What is the fate of the tuber when the whole structure is allowed to germinate? A number of inter-

FIG. 150. Black raspberry, with branches of stolon rooting. After Beal.

esting transformations may be made by various methods of culture of the tubers.[1]

[1] Vöchting, H. Ueber Knollengewächse. 1899.
Vöchting, H. Ueber die Bildung der Knollen. Bibliotheca Botanica. Hft. 4. 1887.

415. Propagation by Means of Runners, Stolons, Offsets, etc.
Make observations on any plant in the greenhouse or fields that
develops lateral trailing branches, and look for buds which may
give rise to a new plant. *Fragaria, Rubus, Ranunculus* and a
large number of other species furnish suit-
able material. Follow the development of
such buds and note the manner of separa-
tion of the plantlets from the parent.
Note also at what season propagation
takes place ; does it coincide with the for-
mation of seeds ? (See Figs. 149, 150.)

Many plants, notably the willows, have
slender twigs, which are easily broken
from the stem and if thrown into moist
soil or water develop roots and form a
new individual.

416. Bulbils of Lysimachia. Note the
formation of shortened branches by *Lysi-*

FIG. 151. Bulbils in upper axils of stem of *Lysimachia terrestris*.

FIG. 152. Germination of bulbil, in which a leafy shoot is produced, and the bulbil
completes its development by becoming a rhizome.

machia terrestris in the autumn on plants growing in the open,
or upon shoots forced from the rhizomes brought into the green-
house at the close of the season. Examine structure of such
branches ; they will be found to consist of a short branch in
which the stele is in an undeveloped condition, with only pro-

toxylem and protophloem present.   Compare transverse section
with that of the normal branches.   Note amount and char-
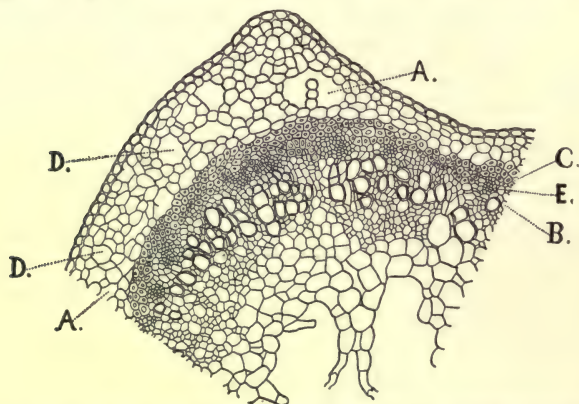
FIG. 153. Cross section of portion of aërial stem of *Lysimachia terrestris*. *A*, large intercellular spaces. *B*, xylem. *C*, bast. *E*, cambium. *D*, glandular ducts.

acter of storage material.   Note specific gravity by placing in
water.   Imbed in moist sand and germinate.   What is the fate
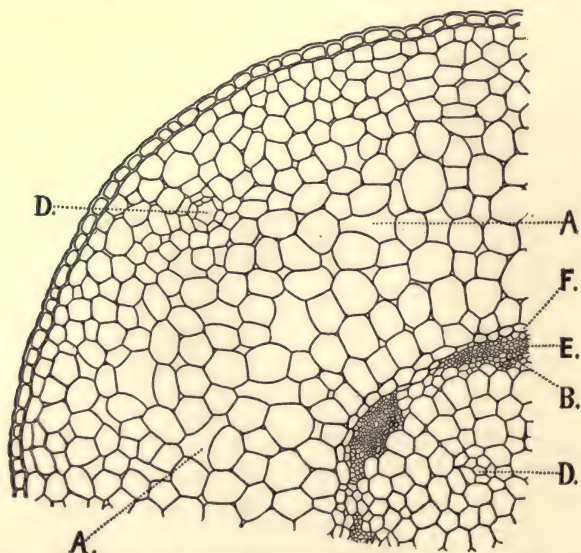
FIG. 154. Cross section of portion of bulbil of *Lysimachia terrestris*. *A*, intercellular spaces. *B*, protoxylem. *E*, procambium. *F*, sheath. *D*, glandular ducts.

of the bulbil? Are juvenile leaf-forms produced? Examine
the scales and epidermis of the bulbil. The bulbil will be found
to complete its arrested development as a stem, becoming the
rhizome or main axis of the new plant[1] (See Figs. 151-154).

417. Reproduction of Lilium by Bulbils. Note the formation of
bulbils in the axils of some of the species of *Lilium*. Germinate
and note fate during the process. Dissect and ascertain the loca-
tion of the growing points. What is the character of the storage
material? Test endurance of the bulbils to desiccation, cold,
heat, and chemicals.

418. Reproduction of Aquatic Plants by Buds. Observe the
death of plants of *Utricularia*, *Philotria*, and *Potamogeton* grow-
ing in ponds or lakes in the autumn. The terminal buds are
seen to be densely clothed with leaves forming a more or less
compact mass that sinks to the bottom after the death of the
main stem. Secure a number of such buds and place in an
aquarium in a temperate house. Note manner of growth and
fate of buds. Here as in *Lysimachia* the bud will be found to
form part of the new plant (Fig. 155).

419. Grafts. Grafting is a special method of propagating cut-
tings much used in horticultural practice for the multiplication
and preservation of special varieties of plants with woody stems.
It may also be used with herbaceous plants, although but little
practical advantage is to be gained from it. It consists essen-
tially in attaching a cutting containing one or more buds, to the
root or stem of another plant in such manner that both the cut-
ting and the stock on which it is placed form a callus, which unites
and develop a series of connecting tissues correspondent to those
of the stock and scion as the cutting is generally termed. After
the two are united the buds of the stock are generally sup-
pressed in practice, and the crown of the plant will be composed
of the branches developed from the scion, although it is possible
to unite a number of the cions of different kinds to a stock and

[1] MacDougal. Vegetative propagation of *Lysimachia terrestris*. Bull. N. Y. Bot.
Gar. **2**: No. 6. 1901.

22

also allow some of the buds of the stock to grow, forming a
crown with many kinds of varieties in it.   Grafting is generally
most successful between closely related plants and may be ac-
complished only when the scion and stock show a structure gen-
erally similar.

A cutting grafted on a stock instead of being cultivated on a
substratum is relieved from the necessity of replacing the root
system, and is furnished with water and mineral salts by a root-
system of comparatively great capacity.   It is this difference in

155
156
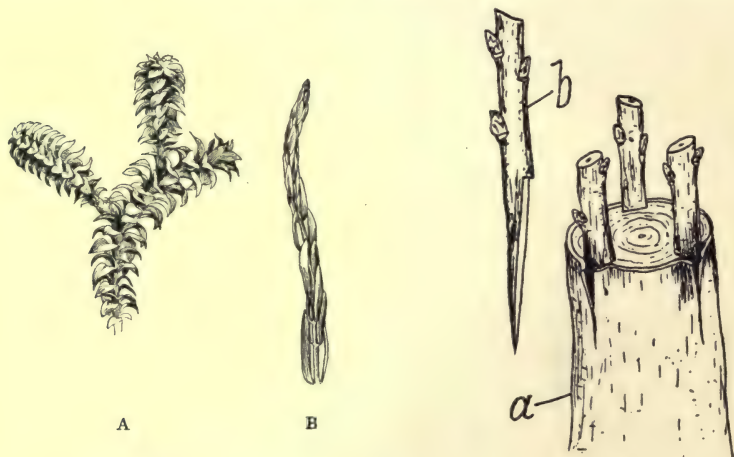
FIG. 155. *A*, winter bud of *Philotria*.   *B*, apex of growing shoot.
FIG. 156. Illustrating crown grafting.   *a*, stock with three scions inserted.   *b*, pre-
pared scion.   After Percival.

amount of nutrition which is chiefly responsible for the differ-
ences between the growth of a grafted cutting and a branch on
the plant from which the cutting was taken.

The union of a scion and stock showing the greatest differences
may be made in herbaceous plants where such unlike forms as
Lycopersicum and *Tradescantia* have been grafted.   The rapidity
with which the union of scion and stock takes place makes these
forms most useful for an experimental study of the subject.[1]

[1] Wright, J. S.   Cell-union in herbaceous grafting.   Bot. Gazette. **18** : 184.
1893.

The extensive technique of various kinds of grafting may be found in practical books on horticulture.[1]

420. Veneer Grafting of Herbaceous Plants. Secure healthy specimens of *Lycopersicum* about 25 cm. in height, or larger, *Solanum tuberosum* of the same size and a number of geraniums.

Make the following grafts : cut a tangental slice from the surface of a part of the stem of the tomato firm enough not to be easily crushed, in such manner that the ring of woody tissue is cut into. Now select a geranium stem of the same size and cut off a section of the stem a few cm. long from which the leaves have been removed with the possible exception of one or part of one. Make a tangental slice on one side of this cutting deep enough so that the wood of the scion and stock, as well as the cambium of both, will be in contact when the scion is applied to the stock. Tie the scion in position with the tissues firmly pressed together by means of soft cords, or raffia fiber. It may also be of advantage to bandage with wet moss or cover the union with a layer of soft wax made of beeswax, resin and lard to prevent

FIG. 157. Transverse section of union of a scion of potato, *P*, to a stock of tomato, *T*. 1, 1, line of contact of the tissues of the two plants. *cm*, cambium. After Wright.

desiccation. Cut away the stock above graft and set the preparation in a cool house for about ten days, then bring into a temperate house. Care must be taken not to disturb the scion during the process of union, and to remove all leaves and branches of he stock below the graft.

Repeat the process, putting scions of tomato on potato and scions of potato on tomato. The greenhouse stock will offer many other examples of suitable material for such experimentation.

[1] Bailey, L. H. The Nursery Book. 1896.

Compare the leaves and flowers formed on grafts with those on the plant from which they were taken.   After union of scion and stock has been accomplished cut transverse and longitudinal sections of the united portions and ascertain the method of union of the tissuesof the scion and stock.

It will be profitable also to make grafts with such woody plants as the apple, rose, and other convenient species.

421. Propagation by Buds Formed on Leaves. A discussion of buds formed on leaves of various ferns has been given in a previous section.   A large number of seed plants are found to bear buds on leaves, among which are *Nasturtium officinale*, and *Cardamine pratensis*.   Still a larger number are capable of developing buds, when mutilated or separated from the shoot.   This capacity is widely prevalent among succulents.

422. Leaves of Begonia. Cut off a separate leaf of any convenient species of *Begonia*, or of *Bryophyllum* and press down on sand in moist chamber.   Cut another leaf into fragments and insert the edges in the sand.   Note the formation of buds and the manner in which new plants arise.   Repeat with any succulent plant.

423. Formation of Tubers and Plants by Leaves of Gloxinia. Cut vigorous leaves of *Gloxinia* from the stem and insert the petiole deeply in moist sand under a bell-jar.   Note the formation of roots from the leaf cutting and the development of a tuber. Follow the course of the leaf; does it form a part of the new plant?   Repeat with leaves of *Boussingaltia baselloides* (Fig. 158).[1]

424. Propagation of Apios tuberosa. Make cuttings from vines of *Apios* which shall include a leaf and a short section of stem to which the petiole is attached.   Imbed the stem and base of the petiole in moist sand and cover with a bell-glass.   Note the course of growth of the new individual : are juvenile leaf forms to be seen?

425. Propagation by Flowering Branches. The replacement of flowering branches by propagative buds is exhibited by a number of alpine forms inclusive of *Poa alpina*, *Poa bulbosa*, and various

[1] Vöchting, H. Physiologie der Knollengewächse. Jahrb. Wiss. Bot. **24** : 54. 1899.

species of *Aira*, and *Festuca*, and *Saxifraga*, and *Polygonum vivi-
parum*. Perhaps the most available plants for the observation
of this fact is to be found in the various species of *Allium, Cor-
dyline viviparum*, and *Primula Forbesii* in which buds or leafy
shoots appear among the branches
of the inflorescence, and are easily
detachable and propagate the species.[1]

A hybrid between *Begonia incar-
nata*, and *B. manicata*, which seems
to be almost identical with a species
from South America known as *B.
phyllomaniaca*, shares with the latter
species the capacity for producing
buds in great profusion over the en-
tire shoot, including the branches of
the inflorescence. These may be
separated and give rise to new plants.

**426. General Nature and Relations
of Reproduction.** The reproduction
of the plant by either monogenetic, or
digenetic spores, may be regarded as
special forms of growth, dependent
upon, and closely connected with
ordinary vegetative growth. So long
as conditions favorable to vegetative
growth are prevalent, reproductive

FIG. 158.   Leaf cutting of
Boussingaltia baselloides. The
root developed from the leaf has
become tuberous. After Vöchting.

processes are not carried on so freely, as when adverse in-
tensities of various trophic factors prevail. This is noticeably
true of the simpler organisms, and is richly illustrated by the
activities of the higher plants. The simple fact does not always
appear in the history of any given species however, since the
production of digenetic reproductive bodies may have become a
rhythmical proceeding that is carried out in the individual regard-
less of the surroundings. Reproductive bodies formed either

[1] MacDougal.   Nature and Work of Plants. p. 135.   1900.   New York.
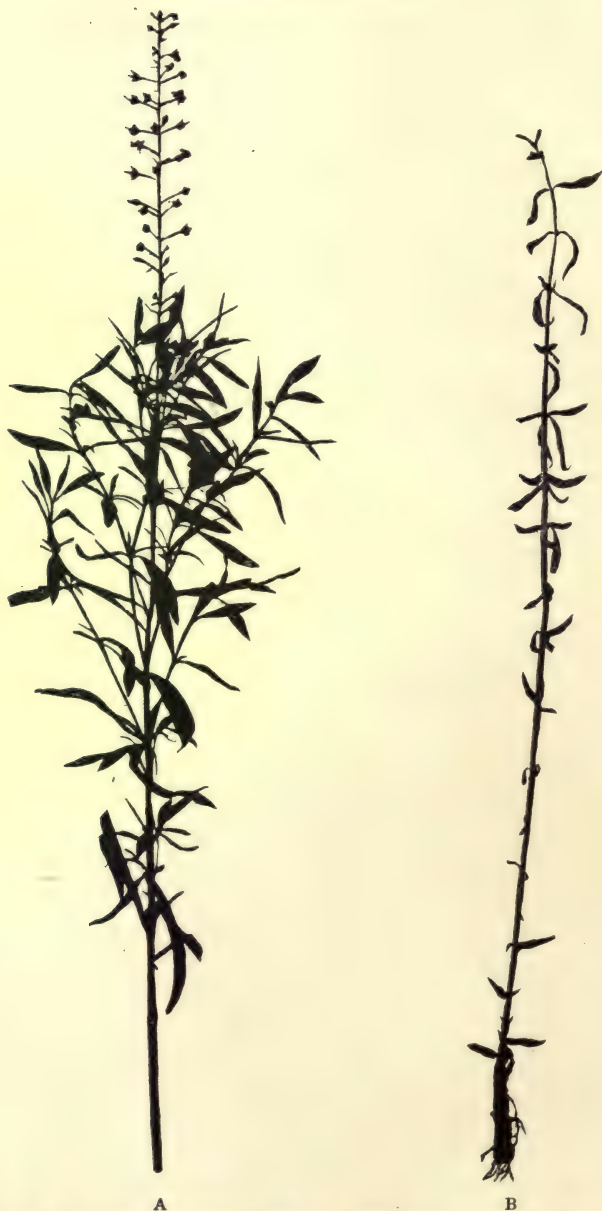
FIG. 159. *A*, Normal flowering shoot of *Lysimachia terrestris*. *B*, aërial shoot grown in diffuse light, and dry atmosphere, with all branches replaced by bulbils.

digenetically, or monogenetically, are generally able to endure much greater ranges of adverse conditions than portions of the vegetative body, or any somatic product.

The constructive process of reproduction may be carried on however, within a much narrower range of trophic conditions than the purely vegetative processes, the range between the maximum and minimum of any given factor being much smaller than for growth. The more important factors concerning reproduction are the chemical composition of the substratum, composition and pressure of the air, and water, oxygen, temperature, light, and perhaps electric currents. A variation of any one of these factors may be the cause of inhibiting the formation of spores by any one method, and may set in action the mechanism by which spores of a different origin are produced, or may cause a transition from purely somatic propagation, to digenetic reproduction, or vice versa. This may be illustrated by the following.

427. Influence of External Conditions upon Vaucheria. If vigorous specimens *Vaucheria terrestris* are removed from natural conditions on moist soil and cultivated in strong sugar solutions no formation of spores will be shown. Similar plants kept in small aquaria containing 1–3 per-cent. solutions of sugar, at room temperatures, will produce sexual organs in one to two weeks. If specimens are cultivated in a nutrient solution consisting of 100 cc. distilled water, .05 g. ammonium nitrate, .02 g. magnesium sulphate, .02 g. magnesium phosphate, and .01 g. calcic chloride for a week or ten days and then removed to distilled water and placed in the dark, zoöspores will be formed in a few days, perhaps within twenty-four hours.[1]

[1] Klebs, G. Ueber einige Probleme der Physiologie der Fortpflanzung. Jena. 1895.

Klebs, G. Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896.

Klebs, G. Zur Physiologie der Fortpflanzung einiger Pilzen. Jahrb. Wiss. Bot. **35** : Hft. 2. 1900.

Mobius, M. Zur Lehre von der Fortpflanzung. Jena. 1897.

Livingston, B. E. On the nature of the stimulus which causes the change of form in polymorphic green algae. Bot. Gazette. **30** : 289. 1901.

APPENDIX

Metric into British.

μ	in.	mm.	in.	mm.	in.
1	.000039	1	.039370	56	2.204726
2	.000079	2	.078740	57	2.244096
3	.000118	3	.118110	58	2.283467
4	.000157	4	.157480	59	2.322837
5	.000197	5	.196851	60	2.362207
6	.000236	6	.236221		
7	.000276	7	.275591		
8	.000315	8	.314961	61	2.401577
9	.000354	9	.354331	62	2.440947
10	.000394	10	.393701	63	2.480317
				64	2.519687
11	.000433			65	2.559057
12	.000472	11	.433071	66	2.598427
13	.000512	12	.472441	67	2.637798
14	.000551	13	.511811	68	2.677168
15	.000591	14	.551182	69	2.716538
16	.000630	15	.590552	70	2.755908
17	.000669	16	.629922		
18	.000709	17	.669292		
19	.000748	18	.708662	71	2.795278
20	.000787	19	.748032	72	2.834648
		20	.787402	73	2.874018
21	.000827			74	2.913388
22	.000866			75	2.952758
23	.000906	21	.826772	76	2.992129
24	.000945	22	.866142	77	3.031499
25	.000984	23	.905513	78	3.070869
26	.001024	24	.944883	79	3.110239
27	.001063	25	.984253	80	3.149609
28	.001102	26	1.023623		
29	.001142	27	1.062993		
30	.001181	28	1.102363	81	3.188979
		29	1.141733	82	3.228349
31	.001220	30	1.181103	83	3.267719
32	.001260			84	3.307089
33	.001299			85	3.346460
34	.001339	31	1.220473	86	3.385830
35	.001378	32	1.259841	87	3.425200
36	.001417	33	1.299214	88	3.464570
37	.001457	34	1.338584	89	3.503940
38	.001496	35	1.377954	90	3.543310
39	.001535	36	1.417324		
40	.001575	37	1.456694		
		38	1.496064	91	3.582680
41	.001614	39	1.535434	92	3.622050
42	.001654	40	1.574805	93	3.661420
43	.001693			94	3.700791
44	.001732			95	3.740161
45	.001772	41	1.614175	96	3.779531
46	.001811	42	1.653545	97	3.818901
47	.001850	43	1.692915	98	3.858271
48	.001890	44	1.732285	99	3.897641
49	.001929	45	1.771655		
50	.001969	46	1.811025		
		47	1.850395	dm.	in.
60	.002362	48	1.889765	1	3.9370113
70	.002756	49	1.929136	2	7.8740226
80	.003150	50	1.968506	3	11.8110339
90	.003543			4	15.7480452
100	.003937	51	2.007876	5	19.6850565
200	.007874	52	2.047246	6	23.6220678
300	.011811	53	2.086616	7	27.5590791
400	.015748	54	2.125986	8	31.4960904
500	.019685	55	2.165356	9	35.4331017
600	.023622				
700	.027559				
800	.031496			1 meter	= 3.2808428 ft.
900	.035433				= 1.09361426 yd.
1000	(= 1 mm.)				

British into Metric.

in.	mm.	in.	mm.
1	25.399978	1/20	1.269999
2	50.799956	1/21	1.209523
3	76.199934	1/22	1.154544
4	101.599912	1/23	1.104347
5	126.999890	1/24	1.058332
6	152.399868	1/25	1.015999
7	177.799846	1/30	.846666
8	203.199824	1/35	.725714
9	228.599802	1/40	.634999
10	253.999780	1/45	.564444
11	279.399758	1/50	.508000
		1/55	.461818
1 ft.	304.799736	1/60	.423333
1 yd.	914.399208	1/65	.390769
in.	mm	1/70	.362857
1/2	12.699989	1/75	.338466
1/3	8.466659	1/80	.317500
2/3	16.933319	1/85	.298823
1/4	6.349994	1/90	.282222
3/4	19.049983	1/95	.267368
1/5	5.079996	1/100	.254000
2/5	10.159991	1/150	.169333
3/5	15.239987	1/200	.127000
4/5	20.319982	1/250	.101600
1/6	4.233330	1/300	.084667
5/6	21.166648	1/350	.072571
1/7	3.628568	1/400	.063500
1/8	3.174997	1/450	.056444
3/8	9.521992	1/500	.050800
5/8	15.874986	1/550	.046182
7/8	22.224980	1/600	.042333
1/9	2.822220	1/650	.039077
1/10	2.539998	1/700	.036286
3/10	7.619993	1/750	.033867
7/10	17.779985	1/800	.031750
9/10	22.859980	1/850	.029882
1/11	2.309089	1/900	.028222
1/12	2.116665	1/950	.026737
5/12	10.583324	in.	μ
7/12	14.816654	1/1000	25.399978
11/12	23.283313	1/2000	12.699989
1/13	1.953844	1/3000	8.466659
1/14	1.814284	1/4000	6.349994
1/15	1.693332	1/5000	5.079996
1/16	1.587499	1/6000	4.233330
3/16	4.762496	1/7000	3.628568
5/16	7.937493	1/8000	3.174997
7/16	11.112490	1/9000	2.822220
9/16	14.287487	1/10000	2.539998
11/16	17.462485	1/15000	1.693332
13/16	20.637482	1/20000	1.269999
15/16	23.812479	1/25000	1.015999
1/17	1.494116		
1/18	1.411110		
1/19	1.336841		

TABLES FOR CONVERTING METRIC WEIGHTS AND MEASURES TO U. S. WEIGHTS AND MEASURES.[1]—WEIGHT.

	Milligrams to grains.	Grams to ounces.	Kilograms to ounces avoirdupois.	Ki'ograms to pounds avoirdupois.	Kilograms to ounces Troy.
1	0.0154	0.0353	35.274	2.205	32.151
2	0.0309	0.0705	70.548	4.409	64.301
3	0.0463	0.1058	105.822	6.614	96.452
4	0.0617	0.1411	141.096	8.818	128.603
5	0.0772	0.1764	176.370	11.023	160.754
6	0.0926	0.2116	211.644	13.228	192.904
7	0.1080	0.2469	246.918	15.432	225.055
8	0.1235	0.2822	282.192	17.637	257.206
9	0.1389	0.3175	317.466	19.842	289.357

CAPACITY.

	Cc. to fluid grains.	Cc. to fluid ounces.	Liters to fluid ounces.	Liters to quarts.
1	0.27	0.0338	33.8	1.0567
2	0.54	0.0676	67.6	2.1134
3	0.81	0.1014	101.4	3.1700
4	1.08	0.1353	135.3	4.2267
5	1.35	0.1691	169.1	5.2834
6	1.62	0.2029	202.9	6.3401
7	1.89	0.2367	236.7	7.3968
8	2.16	0.2705	270.5	8.4535
9	2.43	0.3043	304.3	9.5101

U. S. WEIGHTS AND MEASURES TO METRIC SYSTEM.—WEIGHT.[1]

	Grains to milligrams.	Avoirdupois ounces to grams.	Avoirdupois pounds to kilos.	Troy ounces to grams.
1	64.80	28.349	0.4536	31.103
2	129.60	56.699	0.9072	62.207
3	194.40	85.049	1.3608	93.310
4	259.20	113.398	1.8144	124.414
5	323.99	141.748	2.2680	155.517
6	388.79	170.097	2.7216	186.621
7	453.59	198.447	3.1751	217.724
8	518.39	226.796	3.6287	248.828
9	583.19	255.146	4.0823	279.931

CAPACITY.

	Fl. drams to cc.	Fl. ounces to cc.	Quarts to liters.	Gallons to liters.
1	3.70	29.57	0.94636	3.7854
2	7.39	59.15	1.89272	7.5709
3	11.09	88.72	2.83908	11.3563
4	14.79	118.29	3.78543	15.1417
5	18.48	147.87	4.73179	18.9272
6	22.18	177.44	5.67815	22.7126
7	25.88	207.02	6.62451	26.4980
8	29.57	236.59	7.57087	30.2835
9	33.27	266.16	8.51723	34.0689

[1] From Smithsonian Tables, 1897, slightly modified.

COMPARISON OF FAHRENHEIT SCALE WITH CENTIGRADE: $t°$ F. $= \dfrac{5}{9}(t-32)°$C.

Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.
212	100.00	168	75.55	124	51.11	80	26.67	36	2.22
211	99.44	167	75.00	123	50.55	79	26.11	35	1.67
210	98.89	166	74.44	122	50.00	78	25.55	34	1.11
209	98.33	165	73.89	121	49.44	77	25.00	33	0.55
208	97.78	164	73.33	120	48.89	76	24.44	32	0.00
207	97.22	163	72.78	119	48.33	75	23.89	31	− 0.55
206	96.67	162	72.22	118	47.78	74	23.33	30	− 1.11
205	96.11	161	71.67	117	47.22	73	22.78	29	− 1.67
204	95.55	160	71.11	116	46.67	72	22.22	28	− 2.22
203	95.00	159	70.55	115	46.11	71	21.67	27	− 2.78
202	94.44	158	70.00	114	45.55	70	21.11	26	− 3.33
201	93.89	157	69.44	113	45.00	69	20.55	25	− 3.89
200	93.33	156	68.89	112	44.44	68	20.00	24	− 4.44
199	92.78	155	68.33	111	43.89	67	19.44	23	− 5.00
198	92.22	154	67.78	110	43.33	66	18.89	22	− 5.55
197	91.67	153	67.22	109	42.78	65	18.33	21	− 6.11
196	91.11	152	66.67	108	42.22	64	17.78	20	− 6.67
195	90.55	151	66.11	107	41.67	63	17.22	19	− 7.22
194	90.00	150	65.55	106	41.11	62	16.67	18	− 7.78
193	89.44	149	65.00	105	40.55	61	16.11	17	− 8.33
192	88.89	148	64.44	104	40.00	60	15.55	16	− 8.89
191	88.33	147	63.89	103	39.44	59	15.00	15	− 9.44
190	87.78	146	63.33	102	38.89	58	14.44	14	−10.00
189	87.22	145	62.78	101	38.33	57	13.89	13	−10.55
188	86.67	144	62.22	100	37.78	56	13.33	12	−11.11
187	86.11	143	61.67	99	37.22	55	12.78	11	−11.67
186	85.55	142	61.11	98	36.67	54	12.22	10	−12.22
185	85.00	141	60.55	97	36.11	53	11.67	9	−12.78
184	84.44	140	60.00	96	35.55	52	11.11	8	−13.33
183	83.89	139	59.44	95	35.00	51	10.55	7	−13.89
182	83.33	138	58.89	94	34.44	50	10.00	6	−14.44
181	82.78	137	58.33	93	33.89	49	9.44	5	−15.00
180	82.22	136	57.78	92	33.33	48	8.89	4	−15.55
179	81.67	135	57.22	91	32.78	47	8.33	3	−16.11
178	81.11	134	56.67	90	32.22	46	7.78	2	−16.67
177	80.55	133	56.11	89	31.67	45	7.22	1	−17.22
176	80.00	132	55.55	88	31.11	44	6.67	0	−17.78
175	79.44	131	55.00	87	30.55	43	6.11	−10	−23.33
174	78.89	130	54.44	86	30.00	42	5.55	−20	−28.89
173	78.33	129	53.89	85	29.44	41	5.00	−30	−34.44
172	77.78	128	53.33	84	28.89	40	4.44	−40	−40.00
171	77.22	127	52.78	83	28.33	39	3.89		
170	76.67	126	52.22	82	27.78	38	3.33		
169	76.11	125	51.67	81	27.22	37	2.78		

COMPARISON OF CENTIGRADE SCALE WITH FAHRENHEIT: $t°$ C. $=\frac{9}{5}t + 32°$ F.[1]

Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.
+130	+266	+78	+172.4	+54	+129.2	+30	+86.0	+6	+42.8
120	248	77	170.6	53	127.4	29	84.2	5	41.0
100	212.0	76	168.8	52	125.6	28	82.4	4	39.2
99	210.2	75	167.0	51	123.8	27	80.6	3	37.4
98	208.4	74	165.2	50	122.0	26	78.8	2	35.6
97	206.6	73	163.4	49	120.2	25	77.0	1	33.8
96	204.8	72	161.6	48	118.4	24	75.2	0	32.0
95	203.0	71	159.8	47	116.6	23	73.4	— 1	30.2
94	201.2	70	158.0	46	114.8	22	71.6	— 2	28.4
93	199.4	69	156.2	45	113.0	21	69.8	— 3	26.6
92	197.6	68	154.4	44	111.2	20	68.0	— 4	24.8
91	195.8	67	152.6	43	109.4	19	66.2	— 5	23.0
90	194.0	66	150.8	42	107.6	18	64.4	— 6	21.2
89	192.2	65	149.0	41	105.8	17	62.6	— 7	19.4
88	190.4	64	147.2	40	104.0	16	60.8	— 8	17.6
87	188.6	63	145.4	39	102.2	15	59.0	— 9	15.8
86	186.8	62	143.6	38	100.4	14	57.2	—10	14.
85	185.0	61	141.8	37	98.6	13	55.4	—20	— 4.
84	183.2	60	140.0	36	96.8	12	53.6	—30	—22.
83	181.4	59	138.2	35	95.0	11	51.8	—40	—40.
82	179.6	58	136.4	34	93.2	10	50.0		
81	177.8	57	134.6	33	91.4	9	48.2		
80	176.0	56	132.8	32	89.6	8	46.4		
79	174.2	55	131.0	31	87.8	7	44.6		

SOME CONSTANTS CONCERNING AIR.

Weight of one liter of air at the barometric pressure of 760 mm. and the temperature of 0° C., 1.293 grams. Per cent., by weight, of oxygen in pure air under above conditions, 23.18%. Per cent., by weight, of nitrogen (including argon) in pure air under above conditions, 76.82%. Per cent. of CO_2 varying quantity usually taken at 0.03–0.04% HNO_3 and NH_3 present as traces under some conditions.

Amount of water vapor contained in saturated air at the pressure of 760 mm. and at various temperatures.

0°C.	4.835	20°C.	17.118
5	6.761	25	22.796
10	9.330	30	30.039
15	12.712	35	39.187

[1] Smithsonian Tables, 1897.

EXPANSION OF AIR AT DIFFERENT TEMPERATURES

In dealing with enclosed volumes of air at varying tempera-
tures it is convenient to have some idea of the resulting change
of volume.   The table below is based on the quantity $1 + 00367t$
when the pressure is constant and with dry air.   The volume of
air at 0° being unity, its expansion per degree is shown.   In
using such a table without regard to changes of atmospheric
pressure, or variation of water vapor in the air, it must be remem-
bered that the results are not without error.   For proper correc-
tions to be made for these sources of error, any work on gas
analysis may be consulted, but by far the greatest change in
volume is due to the expansion of the air itself, without regard to
ordinary changes of atmospheric pressure, or to moisture.

RELATIVE VALUE OF 1 PART OF AIR AT VARIOUS TEMPERATURES.

0°	1.0000	18°	1.0661
1°	1.0037	19°	1.0697
2°	1.0073	20°	1.0734
3°	1.0110	21°	1.0771
4°	1.0147	22°	1.0807
5°	1.0183	23°	1.0844
6°	1.0220	24°	1.0881
7°	1.0257	25°	1.0917
8°	1.0294	26°	1.0954
9°	1.0330	27°	1.0991
10°	1.0367	28°	1.1028
11°	1.0404	29°	1.1064
12°	1.0440	30°	1.1101
13°	1.0477	31°	1.1138
14°	1.0514	32°	1.1174
15°	1.0587 550	33°	1.1211
16°	1.0624	34°	1.1248
17°	1.0661	35°	1.1284

DENSITY OF OXYGEN (O_2).[1]

Weight of 1 cc. of oxygen in milligrams at 760 mm. baro-
metric pressure, and from 10°–25° C temperature.

10°	1.362	16°	1.326	22°	1.288
11°	1.356	17°	1.320	23°	1.282
12°	1.350	18°	1.314	24°	1.276
13°	1.344	19°	1.307	25°	1.269
14°	1.338	20°	1.301		
15°	1.332	21°	1.295		

[1] Adapted from Chemiker Kalendar, 1893, R Biedermann.

DENSITY OF CARBON DIOXIDE (CO_2).[1]

Weight of 1 cc. CO_2 in milligrams at 760 mm. barometric pressure, and from 10°–25° C. temperature.

10°	1.874	16°	1.825	22°	1.773
11°	1.861	17°	1.816	23°	1.764
12°	1.853	18°	1.808	24°	1.755
13°	1.850	19°	1.799	25°	1.746
14°	1.842	20°	1.791		
15°	1.833	21°	1.782		

For every 10 mm. above or below 760 mm. 0.024 mg. can be added to, or subtracted from, the weight given and a sufficiently accurate result obtained.

ABSORPTION OF CO_2 AND O_2 BY WATER.

One volume of water will absorb at atmospheric pressure the following volumes of the two gases named, these being referred to the density at 0° and 760 mm. pressure.

°C.	CO_2	O_2	Air.
0	1.797	0.0492	0.0247
5	1.450	0.0433	0.0218
10	1.185	0.0385	0.0195
15	1.002	0.0346	0.0179
20	0.901	0.0314	0.0170
25	0.772	0.0287	
30	0.639[2]	0.0265	
40	0.506	0.0232	
50	0.375[2]	0.0208	
100	0.244	0.0169	

Adapted from Smithsonian Tables, 1897, No. 138.

ATMOSPHERIC PRESSURE.

Atmospheric pressure at 760 mm.[3]

For every 10 mm. above or below 760 mm. 0.018 mg. may be added or subtracted from the weight given and a sufficiently accurate result obtained.

Sq. cm. in kilos.	Sq. inch in pounds.
1.0333	14.657

[1] Adapted from Chemiker Kalendar, 1893, R. Biedermann.
[2] Interpolated.
[3] From Landolt and Bornstein.

HEIGHT OF WATER COLUMN REDUCED TO THAT OF MERCURY (BAROMETER)
IN MILLIMETERS.[1]

Aq.	Hg.	Aq.	Hg.	Aq.	Hg.	Aq.	Hg.
1	0.07	29	2.14	57	4.21	85	6.27
2	0.15	30	2.21	58	4.28	86	6.35
3	0.22	31	2.29	59	4.35	87	9.42
4	0.30	32	2.36	60	4.43	88	6.49
5	0.37	33	2.44	61	4.50	89	6.57
6	0.44	34	2.51	62	4.58	90	6.64
7	0.52	35	2.58	63	4.65	91	6.72
8	0.59	36	2.66	64	4.72	92	6.79
9	0.66	37	2.73	65	4.80	93	6.86
10	0.74	38	2.80	66	4.87	94	6.94
11	0.81	39	2.88	67	4.94	95	7.01
12	0.89	40	2.95	68	5.02	96	7.08
13	0.96	41	3.03	69	5.09	97	7.16
14	1.03	42	3.10	70	5.17	98	7.23
15	1.12	43	3.17	71	5.24	99	7.31
16	1.18	44	3.25	72	5.31	100	7.38
17	1.26	45	3.32	73	5.39	200	14.76
18	1.33	46	3.39	74	5.46	300	22.14
19	1.40	47	3.47	75	5.54	400	29.52
20	1.48	48	3.54	76	5.61	500	36.90
21	1.55	49	3.62	77	5.68	600	44.28
22	1.62	50	3.69	78	5.76	700	51.66
23	1.70	51	3.76	79	5.83	800	59.04
24	1.77	52	3.84	80	5.90	900	66.42
25	1.84	53	3.91	81	5.98	1000	73.80
26	1.92	54	3.99	82	6.05		
27	1.98	55	4.06	83	6.13	10.2981	760
28	2.07	56	4.13	84	6.20		

DENSITY AND VOLUME OF WATER AT DIFFERENT TEMPERATURES (ACCORDING TO VOLKMANN).

Volumetric apparatus and hydrometers are usually graduated to be standard at 15° C. Where extreme accuracy is required the following correction may be applied.

Temp. °C.	Weight 1 cc. H_2O in g.	Vol. 1 g. H_2O in cc.	Temp. °C.	Weight 1 cc. H_2O in g.	Vol. 1 g. H_2O in cc.
0°	0.99988	1.00012	50°	0.98817	1.01197
4°	1.00000	1.00000	55°	.98584	1.01436
5°	0.99999	1.00001	60°	.98334	1.01694
10°	0.99974	1.00026	65°	.98071	1.01967
15°	0.99915	1.00085	70°	.97789	1.02261
20°	0.99827	1.00173	75°	.97493	1.02570
25°	0.99714	1.00287	80°	.97190	1.02891
30°	0.99577	1.00425	85°	.96876	1.03225
35°	0.99417	1.00586	90°	.96549	1.03574
40°	0.99236	1.00770	95°	.96208	1.03941
45°	0.99035	1.00974	100°	.95856	1.04323

[1] From Bunsen. Gasometrische Methoden.

THE PREPARATION OF SOLUTIONS OF DIFFERENT CONCENTRATIONS

In the following formula, which is convenient for ordinary purposes, the contraction which follows the mixing of solutions of salts with water is not taken into account. This contraction in the case of inorganic salts is very slight, and even in preparing various "grades" of alcohol for the usual dehydration purposes the following method is sufficiently accurate,

$$V = \frac{V'b}{a} \text{ or } V' = \frac{Va}{b}.$$

Where V equals the volume of the stock solution to be taken, and a its per cent. of concentration, while V' equals the volume to which V is to be diluted to bring it to the desired per cent. b. $V' - V$ will equal the amount of water to be added to V.

Where the specific gravities of the liquids to be mixed are known the following formula may be employed :[1]

If D is the sp. gr. of a solution of the volume V a certain volume x, of the second solution with a sp. gr. of d must be added to bring the resultant mixture to the desired density d'.

Thus

$$x = \frac{V(D - d')}{d' - d} \text{ or } d' = \frac{VD + xd}{V + x} .$$

FREEZING MIXTURES.

Mixed with 100 parts of snow or powdered ice at approximately 0° C. the substances enumerated below will give about the following temperatures :

Sodic carbonate (cryst.) . . . . .20 parts	—	2°C.
Potassic nitrate 13	"	— 3°
Potassic chloride. 30	"	—11°
Ammonic chloride. 25	"	—15°
Sodic chloride. 33	"	—21°
Calcium chloride (cryst.) 143	"	—50°
Sulphuric or nitric acid (dilute) 100	"	—40°

[1] From Chemiker Kalendar. Biedermann.

The solution of ammonic nitrate in an equal weight of water will reduce the temperature of the mixture from about $+ 10°$ to $-15°$.

Liquid carbon dioxide and ether will give a temperature of $-100°$ C.

Liquid hydrogen gives a temperature of $-252°$.

Liquid air will give a temperature of about $-190 - -200°$ C.

VALUE OF 1 CCM. FEHLING'S SOLUTION IN MG. OF VARIOUS SUGARS.

Dextrose	4.753 mg.[1]	5.00 [2]
Levulose	5.144	5.00
Invert sugar	4.941	4.75
Galactose	5.110	——
Milk sugar	6.757	6.78
Maltose	7.780	8.07

TABLE SHOWING PROPORTIONAL VOLUMES OF OXYGEN AND NITROGEN IN VARIOUS VOLUMES OF AIR.[3]

Vol. of Atmos-pheric Air.	100	200	300	400	500	600	700	800	900
Volume of Nitrogen, etc.	79.04	158.09	237.12	316.16	395.20	474.24	553.28	632.32	711.36
Volume of Oxygen.	20.96	41.92	62.88	83.84	104.80	125.76	146.72	167.68	188.64

[1] Soxhlet. Sugars in 1 per cent. sol. Fehling solution undiluted.

[2] Allen. Agric. Chem. 1 : 226.

[3] From Bunsen. Gasometrische Methoden.

OSMOTIC VALUES.[1]

Substance	Formula	Mol. weight	Isosomotic coefficient.		Value of a 1% sol. in relation to 1.0% sol. KNO₃	Concentration is osmotic with decinormal sol. KNO₃	Osmotic pressure of 1 gm. in 100 cc. solution.	
			Found.	Assigned.			Atmosphere.	Cm. Hg.
Cane sugar	$C_{12}H_{22}O_{11}$	342	1.88	2	0.195	5.13	0.69	52.4
Dextrose and laevulose	$C_6H_{12}O_6$	180	1.88	2	0.37	2.70	1.25	99.5
Glycerine	$C_3H_8O_3$	92	1.78	2	0.73	1.39	2.54	193.3
Citric acid	$C_6H_8O_7$	192	2.02	2	0.35	2.88	1.23	93.3
Tartaric acid	$C_4H_6O_6$	150	2.02	2	0.44	2.25	1.57	119.4
Malic acid	$C_4H_6O_5$	134	1.98	2	0.50	2.01	1.76	133.7
Oxalic acid	$C_2H_2O_4$	90		2	0.74	1.35	2.62	199.0
Potassium nitrate	KNO_3	101	3.0	3	0.99	1.01	3.50	266.0
Sodium nitrate	$NaNO_3$	85	3.0	3	1.18	0.85	4.16	316.1
Potassium chloride	KCl	74.5	3.0	3	1.34	0.74	4.77	363.0
Sodium chloride	$NaCl$	58.5	3.0	3	1.71	0.58	6.09	463.2
Ammonium chloride	NH_4Cl	53.5	3.0	3	1.87	0.53	6.67	506.3
Potassium bicitrate	$KH_2C_6H_5O_7$	130	3.05	3	0.77	1.30	2.72	206.7
Potassium oxalate	$K_2C_2O_4$	166	3.93	4	0.80	1.24	2.85	216.7
Potassium sulphate	K_2SO_4	174	3.90	4	0.77	1.30	2.72	206.7
Potassium phosphate, dibasic	K_2HPO_4	174	3.96	4	0.77	1.30	2.72	206.7
Basic potassium tartrate	$K_2C_4H_4O_6$	226	3.99	4	0.59	1.69	2.09	159.0
Basic potassium malate	$K_2C_4H_4O_5$	210	4.11	4	0.63	1.57	2.25	171.1
Potassium citrate	$K_2HC_6H_5O_7$	268	4.08	4	0.50	2.01	1.75	133.6
Basic potassium citrate	$K_3C_6H_5O_7$	306	5.01	5	0.54	1.84	1.92	146.0
Magnesium malate	$MgC_4H_4O_5$	156	1.88	2	0.43	2.35	1.51	114.8
Magnesium sulphate	$MgSO_4$	120	1.96	2	0.56	1.80	1.93	149.2
Magnesium citrate	$Mg_3(C_6H_5O_7)_2$	450	3.88	4	0.30	3.37	1.05	79.7
Magnesium chloride	$MgCl_2$	95	4.33	4	1.40	0.71	4.99	378.4
Calcium chloride	$CaCl_2$	111	4.33	4	1.20	0.83	4.26	323.6
Gum arabic						41.32	0.085	6.5
Dextrin						16.18	0.218	16.6

[1] From Pfeffer's Plant Physiology, Trans. by Ewart, I : 146. 1900.

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